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(54) Title: IMMUNE RESPONSE MODULATORS AND USES THEREFOR

(57) Abstract

The present invention relates to a nucleic acid molecule comprising a nucleotide sequence encoding, or complementary to a sequence encoding, an ovine IL-5 or IL-12 cytokine molecule. The invention further provides recombinant isolated ovine IL-5 and IL-12 polypeptides which are useful as immune response modulators in livestock animals.

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IMMUNE RESPONSE MODULATORS AND USES THEREFOR

The present invention relates generally to recombinant polypeptides having ovine cytokine properties and to genetic sequences encoding same. More particularly, the present invention is directed to recombinant ovine interleukins and specifically interleukin-5 (IL-5) and interleukin-12 (IL-12) and their use as immune response modulators, especially in vaccine compositions.

Bibliographic details of the publications referred to in this specification by author are collected at the end of the description. Sequence Identity Numbers (SEQ ID Nos.) for the nucleotide and amino acid sequences referred to in the specification are defined following the bibliography.

Throughout this specification and the claims that follow, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element or integer or group of elements or integers but not the exclusion of any other element or integer or group of elements or integers.

The rapidly increasing sophistication of recombinant DNA technology is greatly facilitating research into the medical and veterinary fields. Cytokine research is of particular importance, especially as these molecules regulate the proliferation, differentiation and function of a variety of cells such as cells involved in mediating an immune response. Administration of recombinant cytokines or regulating cytokine function and/or synthesis is becoming, increasingly, the focus of medical research into the treatment of a range of disease conditions in humans and animals.

Cytokines are the hormones of the immune system which control and determine the nature of the immune response (Balkwill and Burke, 1989). Examples of cytokines include interleukins which primarily effect the functional activity of the lymphocytes involved in specific cell-mediated and antibody responses; colony stimulating factors which regulate the

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maturity of precursor cells into macrophages, granulocytes, mast cells and lymphocytes which are involved in innate resistance to many pathogens (Metcalf, 1987); the interferons, which, in addition to their direct anti-viral action, also stimulate antibody synthesis, the activity of natural killer cells and the antimicrobial activity of macrophages and neutrophils (Bielefeldt 5 Ohmann *et al.*, 1987). All these molecules have the potential to alter the disease resistance and immune responsiveness of animals to a wide variety of infectious diseases and vaccines.

Much research has been undertaken into the use of cytokines to augment the immune response and to enhance the immunocompetence of the host to eliminate foreign pathogens. 10 However, despite the discovery and availability of a range of cytokines and other secreted regulators of cell function, comparatively few cytokines are used directly or targeted in therapeutic regimens, especially in animals. One reason for this is the pleiotropic nature of many cytokines. For example, interleukin-11 (IL-11) is a functionally pleiotropic molecule capable of inducing multipotential haemopoietin progenitor cell proliferation, enhancing 15 megakaryocyte and platelet formation, stimulating acute phase protein synthesis and inhibiting adipocyte lipoprotein lipase activity.

Another difficulty confronted in cytokine research is that much of the work has been conducted in human and murine systems. As a consequence, far less is known of the role of 20 animal cytokines in the regulation of the immune system.

There is a need, therefore, for a detailed elucidation of the immune response in large animals in order to facilitate an understanding of the effector mechanisms required to confer protection to livestock and other animals of commercial, environmental or domestic importance 25 against various disease conditions. This will also provide more efficacious vaccines and the development of veterinary compositions for livestock animals to protect same against infection especially when the animals are in an immunocompromised state such as stress due to overcrowding and during transport, changes in climate and following early weaning. The commercial importance of such formations, especially in the livestock industry are clearly 30 evident, such as in increased production of meat and wool. In work leading up to the present

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invention, the inventors sought to clone ovine cytokine genes. Two cytokines were targeted, IL-5 and IL-12.

IL-5 is a potent growth promoter of early haemopoietic progenitor cells. It also promotes the generation of cytotoxic cells from thymocytes and murine IL-5 stimulates the production and secretion of IgM and IgA by B cells in synergism with bacterial endotoxins (Sonada *et al.*, 1992). Secretory IgA antibodies directed against specific virulence determinants of infecting organisms play an important role in overall mucosal immunity. IL-5 is also a specific stimulator of eosinophil differentiation as well as a selective chemoattractant 10 and eosinophil activation factor.

IL-12 is a heterodimeric cytokine composed of a 40-kDa subunit (p40) disulfide-linked to a 35-kDa subunit (p35) (Kobayashi *et al* 1989); (Stern *et al* 1990). It induces the production of IFN- γ by T and NK cells, stimulates the proliferation of activated T and NK cells and 15 enhances the specific and non-specific cytolytic lymphocyte responses. Accumulating evidence suggests that the lack of effective protection against infectious pathogens may result from the selective activation of T cells with an aberrant cytokine profile. Generally, protection against intracellular bacteria and viruses requires a Th1-type response. IL-12 is the critical cytokine that drives differentiation of naive cells to the Th1 subset resulting in the Th1-type immune 20 response. Thus, IL-12 plays a vital role in inducing protective effector mechanisms against bacterial and viral infections.

In accordance with the present invention, genetic sequences encoding ovine IL-5 and the 35 kDa and 40 kDa subunits of IL-12 have been cloned. The availability of recombinant 25 forms of these two important cytokines will now permit the development of therapeutic and vaccine compositions to enhance the immunoresponsiveness of host animals.

Accordingly, one aspect of the present invention relates to an isolated nucleic acid molecule comprising a nucleotide sequence encoding, or complementary to a nucleotide 30 sequence encoding, an ovine cytokine or a functional or immunologically interactive

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homologue, analogue or derivative thereof, wherein said ovine cytokine is IL-5 or IL-12 or a polypeptide subunit of IL-12, or is a fusion cytokine between different subunits of IL-12.

Hereinafter references to "IL-12" or "ovine IL-12" shall be taken to include all possible 5 monomeric, dimeric or other multimeric forms comprising the 35 kDa or 40 kDa polypeptide subunits, including heterodimers and homodimers comprising same. References herein to "IL-12" shall also be taken to include all possible fusion cytokines between the 35 kDa and the 40 kDa polypeptide subunits of ovine IL-12. In a particularly preferred embodiment however, references contained herein to "IL-12" indicates a heterodimer formed between the 35 kDa and 10 40 kDa polypeptide subunits.

The nucleotide sequence of the cloned cytokines will most preferably include the sequences set forth in SEQ ID No: 1 or SEQ ID No: 3 for IL-5, SEQ ID No: 5 or SEQ ID No: 7 for the 35 kDa subunit of IL-12, or SEQ ID No: 9 for the 40 kDa subunit of IL-12, or a 15 homologue, analogue or derivative thereof including any single or multiple nucleotide substitutions, deletions and/or additions thereto.

In a related embodiment of the present invention there is provided an isolated DNA molecule which:

- 20 (i) encodes a molecule having interleukin activity;
- (ii) is capable of hybridising under at least medium stringency conditions to one or more of the nucleotide sequences set forth in SEQ ID Nos: 1,3,5,7, or 9 or a complementary sequence or a homologue, analogue or derivative thereof; and
- (iii) wherein said interleukin comprises an amino acid sequence corresponding to all 25 or a part of one or more of the amino acid sequences set forth in SEQ ID Nos: 2,4,6,8, or 10 or having greater than 70% similarity thereto.

In a preferred embodiment, the present invention provides an isolated DNA molecule which:

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- (i) encodes a molecule having IL-5 activity;
- (ii) is capable of hybridising under at least medium stringency conditions to all or part of SEQ ID NO: 1 or SEQ ID NO: 3, or a complementary sequence thereof; and
- (iii) said interleukin comprises an amino acid sequence corresponding to SEQ ID No: 5 or SEQ ID No: 4 or having 70% or greater similarity thereto.

In a related preferred embodiment the present invention provides an isolated DNA molecule which:

- (i) encodes a molecule having IL-12 activity;
- (ii) is capable of hybridising under at least medium stringency conditions to one or more of the nucleotide sequences set forth in SEQ ID NO: 5 or SEQ ID NO: 7 or SEQ ID NO: 9 or a complementary form or a homologue, analogue or derivative thereof; and
- (iii) wherein said interleukin comprises an amino acid sequence corresponding to all or a part of one or more of the amino acid sequences set forth in SEQ ID No: 6 or SEQ ID No: 8 or SEQ ID No: 10 or having greater than 80% similarity thereto.

These embodiments of the present invention are not intended to cover nor do they cover human or mouse IL-5 or IL-12.

For the purposes of defining the levels of stringency, reference can conveniently be made to Maniatis *et al* (1982) at pages 387-389 which are incorporated herein by reference where the washing step at paragraph 11 is considered herein to be high stringency. A high stringency wash is defined herein to be 0.1-0.2xSSC, 0.1% w/v SDS at 55-65°C for 20 minutes 25 and a medium level of stringency is considered herein to be 2xSSC, 0.1% w/v SDS at ≥ 45°C for 20 minutes. The alternative conditions are applicable depending on concentration, purity and source of nucleic acid molecules.

In a more particularly preferred embodiment, the present invention provides an isolated 30 nucleic acid molecule which encodes or is complementary to a nucleic acid molecule which

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encodes an ovine IL-5 and having a nucleotide sequence substantially as set forth in SEQ ID NO: 1 or SEQ ID NO: 3, or a homologue, analogue or derivative thereof. In another embodiment, the present invention provides an isolated nucleic acid molecule which encodes, or is complementary to a nucleic acid molecule which encodes a 35 kDa subunit of ovine IL-12
5 and having a nucleotide sequence substantially as set forth in SEQ ID NO: 5 or SEQ ID NO: 7, or a homologue, analogue or derivative thereof. In yet another embodiment, the present invention provides an isolated nucleic acid molecule which encodes or is complementary to a nucleic acid molecule which encodes a 40 kDa subunit of ovine IL-12 and having a nucleotide sequence substantially as set forth in SEQ ID NO: 9 or a homologue, analogue or derivative
10 thereof.

The term "homologue" as used hereinafter, in relation to a variant genetic sequence, refers to a gene which encodes a polypeptide which retains its function as an interleukin molecule or subunit of same, although said polypeptide may contain amino acid substitutions,
15 deletions and/or additions. The term "homologue" in relation to a variant polypeptide refers to a polypeptide containing amino acid substitutions, amino acid deletions and/or amino acid additions which do not affect the function of the polypeptide. Furthermore, amino acids may be replaced by other amino acids having similar properties, for example hydrophobicity, hydrophilicity, hydrophobic moment or antigenicity, and so on. The present invention clearly
20 extends to homologues of ovine IL-5 or IL-12 genetic sequences.

The term "analogue" as used hereinafter in reference to a nucleic acid molecule, shall be taken to refer to a variant genetic sequence which is functionally equivalent to a genetic sequence which encodes or is complementary to a genetic sequence which encodes an ovine IL-
25 5 or ovine IL-12 polypeptide, but which contains certain non-naturally occurring or modified residues. Similarly, the term "analogue" when used in relation to a polypeptide molecule shall be taken to refer to a variant polypeptide which is functionally equivalent to an ovine IL-5 or an ovine IL-12 polypeptide, but which contains certain non-naturally occurring or modified residues.

Hereinafter, reference to "derivatives" includes mutants, parts or fragments of genetic sequences which encode or are complementary to genetic sequences which encode ovine IL-5 or IL-12 polypeptide subunits. The term "derivative" in relation to an ovine IL-5 or IL-12 polypeptide shall be taken to refer hereinafter to mutants, parts or fragments of the complete 5 IL-5 or IL-12 polypeptide subunits comprising the functional ovine interleukin. It is understood by the skilled person in the art that a "derivative" of a nucleic acid molecule or a polypeptide molecule may not have the same physiological activity as the genetic sequence or polypeptide from which it was derived, however it is useful in the isolation of related genetic sequences or polypeptides, or in modifying gene expression, for example by antisense or ribozyme 10 technology, or in the production of useful immunoreactive molecules, for example the production of useful subunit vaccines.

In accordance with the present invention, by "nucleic acid molecule" is meant a single or double stranded sequence of ribonucleotides or deoxyribonucleotides which encode, or are 15 complementary to a sequence which encodes, an ovine IL-5 and/or IL-12 or their derivatives. The nucleic acid molecule may be genomic DNA, cDNA or a synthetic DNA sequence or a derivative thereof. The derivatives may be functional in that they exhibit at least one property or function attributed to IL-5 or IL-12 or are immunologically interactive with antibodies to at least one region of IL-5 or IL-12. The nucleic acid molecule of the present invention is 20 generally in isolated form but the present invention extends to the nucleic acid molecule integrated into a genome or other nucleic acid molecule.

A further aspect of the present invention provides a genetic construct comprising a nucleic acid molecule which encodes or is complementary to a nucleic acid molecule which 25 encodes an ovine IL-5 or ovine IL-12 polypeptide or a homologue, analogue or derivative thereof.

In a particularly preferred embodiment, the present invention provides a genetic construct comprising at least one of the nucleotide sequences set forth in SEQ ID Nos: 1,3,5,7 30 or 9 or a homologue, analogue or derivative thereof.

The genetic constructs of the present invention are particularly useful for the production of recombinant cytokine molecules encoded therein, when introduced into a cell line and under conditions suitable for gene expression to occur. Such conditions will depend upon the cell line and the expression vector used in each case and would be well-known to the person skilled in the art.

Any number of expression vectors can be employed depending on whether expression is required in a eukaryotic or prokaryotic cell. Furthermore, it is well known in the art that the promoter sequence used in the expression vector will also vary depending upon the level of expression required and whether expression is intended to be constitutive or regulated. Examples of eukaryotic cells contemplated herein include mammalian, yeast, insect or plant cells and examples of prokaryotes include *Escherichia coli*, *Bacillus* sp. and *Pseudomonas* sp. Typical promoters suitable for expression in bacterial cells such as *E. coli* include, but are not limited to, *tac* promoter, the *lacZ* promoter, or the phage lambda λ_L or λ_R promoters.

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A still further aspect of the present invention provides a recombinant isolated ovine IL-5 or IL-12 polypeptide or a homologue, analogue or derivative thereof. By "recombinant cytokine" or related term "recombinant molecule" is meant a glycosylated or unglycosylated polypeptide molecule, with or without other associated molecules (eg. lipids) produced by recombinant means such as presence of a DNA molecule in an expression vector in the correct reading frame relative to a promoter and introducing the resultant recombinant expression vector into a suitable host and growing said host under conditions appropriate for expression and, if necessary, transportation of the recombinant protein or its derivative from said host and then purifying the recombinant molecule.

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In a particularly preferred embodiment of the present invention, there is provided a recombinant polypeptide comprising a sequence of amino acids which is substantially the same as the amino acid sequence set forth in any one or more of SEQ ID Nos: 2, 4, 6, 8, or 10, or is at least 70% identical to same. The present invention extends to any derivatives of ovine IL-5 or IL-12 polypeptides set forth in SEQ ID NOS. 2, 4, 6, 8 or 10.

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Derivatives of ovine IL-5 and IL-12 include single or multiple amino acid substitutions, deletions and/or additions to the molecule. Conveniently, these are prepared by first making single or multiple nucleotide substitutions, deletions and/or additions to the nucleic acid molecule encoding the ovine cytokine. Alternatively, once the amino acid sequence is known,
5 ~~amino acids can be chemically added by established techniques and in any sequence required~~ to give the desired mutant. All such derivatives are encompassed by the present invention.

Amino acid insertional derivatives of the ovine cytokines of the present invention include amino and/or carboxyl terminal fusions as well as intra-sequence insertions of single 10 or multiple amino acids. Insertional amino acid sequence variants are those in which one or more amino acid residues are introduced into a predetermined site in the protein although random insertion is also possible with suitable screening of the resulting product. Deletional variants are characterised by the removal of one or more amino acids from the sequence. Substitutional amino acid variants are those in which at least one residue in the sequence has 15 been removed and a different residue inserted in its place. Typical substitutions are those made in accordance with Table 1.

Where a derivative ovine cytokine is produced by amino acid substitution, the amino acids are generally replaced by other amino acids having like properties, such as 20 hydrophobicity, hydrophilicity, electronegativity, bulky side chains and the like. Amino acid substitutions are typically of single residues. Amino acid insertions will usually be in the order of about 1-10 amino acid residues and deletions will range from about 1-20 residues. Preferably, deletions or insertions are made in adjacent pairs, i.e. a deletion of two residues and a corresponding insertion of two residues.

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TABLE 1
Suitable residues for amino acid substitutions

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	Original Residue	Exemplary Substitutions
10	Ala	Ser
	Arg	Lys
	Asn	Gln; His
	Asp	Glu
	Cys	Ser
	Gln	Asn; Glu
15	Glu	Asp
	Gly	Pro
	His	Asn; Gln
	Ile	Leu; Val
	Leu	Ile; Val
20	Lys	Arg; Gln; Glu
	Met	Leu; Ile; Val
	Phe	Met; Leu; Tyr
	Ser	Thr
	Thr	Ser
25	Trp	Tyr
	Tyr	Trp; Phe
	Val	Ile; Leu; Met

For convenience and by way of shorthand notation, reference herein to ovine cytokine IL-5 or IL-12 includes reference to any derivatives thereof as contemplated above.

The amino acid variants referred to above may be readily made using synthetic peptide techniques well known in the art, such as solid phase peptide synthesis and the like, or by recombinant DNA manipulations. Techniques for making substitution mutations at predetermined sites in DNA having known or partially known sequence are well known and 5 include, for example, M13 mutagenesis. The manipulation of DNA sequence to produce variant proteins which manifest as substitutional, insertional or deletional variants are conveniently described, for example, in Sambrook *et al* (1989).

Other examples of recombinant or synthetic mutants and derivatives of the ovine 10 cytokines of the present invention include single or multiple substitutions, deletions and/or additions of any molecule associated with the enzyme such as carbohydrates, lipids and/or proteins or polypeptides.

The recombinant ovine IL-5 and IL-12 molecules contemplated herein will find 15 particular application in the intensive livestock industries such as the live animal export trade, feed-lots and intensive rearing industries. Animals in close containment are subjected to greater environmental challenge with infectious diseases, particularly respiratory infections and are more prone to the immunodepressive effects of stress leading to higher susceptibility to opportunistic pathogens.

20

Accordingly, in a further aspect of the present invention there is provided a method for the treatment and/or prophylaxis of a livestock animal exposed to or infected with a pathogenic organism, said method comprising administering to said animal an immunoresponsive effective amount of ovine IL-5 and/or ovine IL-12 or a homologue, analogue or derivative thereof for 25 a time and under conditions sufficient to maintain, stimulate or enhance the immunoresponsiveness of said animal.

Preferably, the ovine cytokine is a recombinant molecule. The term "livestock animal" extends to sheep, horses, pigs, cows, donkeys, emus, ostriches, alpacas, camels, deer, goats, 30 amongst other animals, provided that the ovine cytokines are effective in those animals.

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Particularly preferred livestock animals are sheep and cows.

Another important application of the cytokines of the present invention is as natural adjuvants for vaccines, particularly for subunit vaccines produced by recombinant DNA technology. In accordance with the present invention, IL-5 and/or IL-12 are used in vaccines to enhance the immunogenicity of antigens, particularly in subunit vaccines. Advances in slow-release technology and the development of live non-pathogenic bacteria and viruses as delivery vectors for these molecules will ensure their cost-effectiveness in sheep and cattle. They may also be used as in nucleic acid vaccination. Accordingly, the present invention extends to a method of enhancing and/or stimulating an immune response to one or more antigens in an animal, said method comprising administering to said animal an immunoresponsive effective amount of IL-5 and/or IL-12.

In a related embodiment, there is contemplated a vaccine comprising an antigen and recombinant ovine IL-5 and/or IL-12 or their derivatives. The vaccine may also comprise one or more pharmaceutically acceptable carriers and/or diluents. The carriers and/or diluents are also required to be acceptable for veterinary use.

The ovine IL-5 and/or IL-12 may also be delivered by genetic means. For example, the recombinant ovine IL-5 and/or IL-12 may be encoded by a genetic construct present in a delivery system such as a virus, yeast, bacterium, protozoan, insect or mammalian cell. The presence of such a delivery system in a target animal will enable delivery of the recombinant ovine cytokine.

According to this embodiment, there is provided a genetic construct comprising a first nucleotide sequence encoding ovine IL-5 or ovine IL-12 or their derivatives and a second nucleotide sequence defining a delivery vehicle. The delivery vehicle is capable of replication in a delivery cell such as a bacterial, yeast, insect, a protozoan animal or a mammalian cell. Generally, the delivery cells would not in normal use be harmful or pathogenic to the target animal. Conveniently, attenuated delivery cells are employed. Particularly useful delivery cells

are bacterial cells, attenuated viruses and particularly suitable delivery vectors are recombinant viral and bacterial vectors.

For example, an attenuated infectious virus is used as a live vaccine. The genetic sequence encoding ovine IL-5 and/or IL-12 or their derivatives is cloned into the viral sequence and the recombinant virus used to infect target animals. The recombinant virus causes infection and replicates in the animal cells resulting in production of the recombinant cytokine. The infecting recombinant virus may subsequently be eliminated after production of an immunomodulating effective amount of the cytokine. A similar protocol is adopted with live bacterial carriers. Alternatively, a recombinant viral vector may be used. A viral vector provides a modified virus capable of infecting a cell but not replicating therein. A viral vector provides a means of introducing a genetic sequence which is transiently capable of expression into the desired cytokine. An "immunomodulating effective amount" is an amount of cytokine sufficient to effect immunomodulation in the target animal, i.e. to enhance the ability of the immune system to develop an effective immune response or to enhance the immunocompetence of the animal or immunogenicity of an antigen which may also be expressed in the genetic vector.

The present invention provides an opportunity to enhance an immune response in animals and in particular livestock animals (such as those described above) by the administration of an ovine IL-5 and/or IL-12 or their derivatives either directly or via their genetic sequences. This is of particular importance since most subunit and synthetic peptide vaccines are only weakly antigenic. The administration of the cytokines may be alone, in combination with an antigen or as a fusion molecule. Administration may be via an attenuated virus, recombinant viral vector nucleic acid vaccine or bacterial vector or may be by administration of the cytokine by, for example, injection or oral ingestion (e.g. in medicated food material).

The present invention extends to a veterinary pharmaceutical composition for use in livestock animals such as to enhance the immune system or accelerate its maturation or improve

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its immunocompetence or to facilitate immunomodulation in said animals, said composition comprising recombinant ovine IL-5 and/or IL-12 or their derivatives, recombinant ovine IL-5 and/or IL-12 fused to an antigen or to each other with or without antigen or genetic sequences encoding same in suitable delivery vehicles. Preferably, where the composition comprises a 5 recombinant cytokine, the composition is injected or orally administered. Where the composition comprises genetic material, it is administered as part of a viral vector, live viral vector, live bacterial vector or nucleic acid vaccine.

Conditions in livestock animals for which treatment might be required include infectious 10 disease, cancer, immunosuppression, allergy and to enhance or suppress reproductive systems. Conditions would also include situations where animals are in an immunocompromised state such as during or following stress, due to overcrowding and transport process, changes in climate and early weaning. The administration of the cytokine molecules may also promote growth and/or early maturation. The animal to be treated and the cytokine in the composition 15 might be "homologous" in the sense that both are of the same species, i.e. both ovine species or may be "heterologous" where the ovine cytokine is effective in another animal. The compositions may also contain other active molecules such as antibiotics or antigen molecules. Combinations of cytokine molecules with antigen molecules may increase the efficacy of vaccines.

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The present invention, therefore, provides a veterinary pharmaceutical composition comprising an immunomodulatingly effective amount of ovine IL-5 and/or IL-12 or their derivatives or genetic sequences capable of expressing same and one or more carriers and/or diluents acceptable for veterinary use.

25

The active ingredient(s) of the pharmaceutical composition is/are contemplated to exhibit excellent activity in stimulating, enhancing or otherwise facilitating an immune response in an animal species and in particular a livestock animal when administered in an amount which depends on the particular case. The variation depends, for example, on the cytokine and, in 30 some cases, the antigen involved in stimulating the immune response. For example, from about

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0.5 µg to about 100 µg of a particular cytokine which may be combined with other cytokines, per kilogram of body weight per day may be required. Dosage regimen may be adjusted to provide the optimum therapeutic response. For example, several divided doses may be administered in one or more of daily, weekly or monthly or in other suitable time intervals or 5 the dose may be proportionally reduced as indicated by the exigencies of the situation. The active compound may be administered by injection or by oral ingestion in any convenient manner or may be administered via a genetic sequence such as in a viral or bacterial vector or a nucleic acid vaccine.

10 The active compounds may also be administered in dispersions prepared in glycerol, liquid polyethylene glycols, and/or mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms.

15 The pharmaceutical forms suitable for parenteral administration include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. In all cases the form must be sterile and must be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of 20 microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), suitable mixtures thereof, and vegetable oils. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants.

25 The prevention of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example.

Sterile injectable solutions are prepared by incorporating the active compounds in the required amount in the appropriate solvent with various of the other ingredients enumerated above, as required, followed by filter sterilisation. Generally, dispersions are prepared by incorporating the various sterilised active ingredient(s) into a sterile vehicle which contains the 5 basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and the freeze-drying technique which yield a powder of the active ingredient plus any additional desired ingredient from previously sterile-filtered solution thereof.

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Carriers and/or diluents suitable for veterinary use include any and all solvents, dispersion media, aqueous solutions, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent 15 is incompatible with the active ingredient, use thereof in the composition is contemplated. Supplementary active ingredients can also be incorporated into the compositions. The latter is particularly contemplated as far as the present invention extends to multivalent vaccines or multi-component cytokine molecules.

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The pharmaceutical veterinary compositions of the present invention may comprise in addition to IL-5 and/or IL-12 or their derivatives, one or more other active compounds such as antigens and/or immune stimulating compounds.

The cytokine may also be delivered by a live delivery system such as using a bacterial 25 expression system to express the cytokine protein in bacteria which can be incorporated into gut flora. Alternatively, a viral expression system can be employed or incorporated into a BCG vaccine. In this regard, one form of viral expression is the administration of a live vector generally by spray, feed or water where an infecting effective amount of the live vector (e.g. virus or bacterium) is provided to the animal. Another form of viral expression system is a 30 non-replicating virus vector which is capable of infecting a cell but not replicating therein. The

non-replicating viral vector provides a means of introducing genetic material for transient expression into a cytokine. The mode of administering such a vector is the same as a live viral vector.

5 The present invention extends to antibodies raised against ovine IL-5 or IL-12. The antibodies may be monoclonal or polyclonal and may be used for developing enzyme-immunosorbent assays for the rapid diagnosis of infectious diseases of livestock animals. According to this embodiment, there is provided an antibody preparation comprising antibodies or derivatives thereof, immunointeractive with either IL-5 or IL-12 or derivatives thereof.

10

Immunoassays are useful in detecting the presence of IL-5 and/or IL-12 in a target animal.

15 A wide range of immunoassay techniques may be such as those described in US Patent Nos. 4,016,043, 4,424,279 and 4,018,653. These methods may be employed for detecting IL-5 and/or IL-12. By way of example only, an IL-5 or IL-12-specific antibody is immobilised onto a solid substrate to form a first complex and a biological sample from an animal to be tested for the presence of IL-5 or IL-12 brought into contact with the bound molecule. After a suitable 20 period of incubation, for a period of time sufficient to allow formation of an antibody-IL-5/IL-12 secondary complex, a second IL-5/IL-12 antibody labelled with a reporter molecule capable of producing a detectable signal is then added and incubated, allowing sufficient time for the formation of a tertiary complex of antibody-IL-5/IL-12-antibody. Any unreacted material is washed away, and the presence of the tertiary complex is determined by observation of a signal 25 produced by the reporter molecule. The results may either be qualitative, by simple observation of the visible signal or may be quantitated by comparison with a control sample containing known amounts of hapten. Variations of this assay include a simultaneous assay, in which both sample and labelled antibody are added simultaneously to the bound antibody, or a reverse assay in which the labelled antibody and sample to be tested are first combined, incubated and 30 then added simultaneously to the bound antibody. These techniques are well known to those

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skilled in the art, and the possibility of minor variations will be readily apparent. The antibodies used above may be monoclonal or polyclonal.

The solid substrate is typically glass or a polymer, the most commonly used polymers 5 being cellulose, polyacrylamide, nylon, polystyrene, polyvinyl chloride or polypropylene. The solid supports may be in the form of tubes, beads, discs or microplates, or any other surface suitable for conducting an immunoassay. The binding processes are well-known in the art and generally consist of cross-linking covalently binding or physically adsorbing the molecule to the insoluble carrier.

10

By "reporter molecule", as used in the present specification, is meant a molecule which, by its chemical nature, produces an analytically identifiable signal which allows the detection of antigen-bound antibody. Detection may be either qualitative or quantitative. The most commonly used reporter molecule in this type of assay are either enzymes, fluorophores or 15 radionuclide containing molecules (i.e. radioisotopes). In the case of an enzyme immunoassay, an enzyme is conjugated to the second antibody, generally by means of glutaraldehyde or periodate. As will be readily recognised, however, a wide variety of different conjugation techniques exist which are readily available to one skilled in the art. Commonly used enzymes include horseradish peroxidase, glucose oxidase, β -galactosidase and alkaline phosphatase, 20 amongst others. The substrates to be used with the specific enzymes are generally chosen for the production, upon hydrolysis by the corresponding enzyme, of a detectable colour change. It is also possible to employ fluorogenic substrates, which yield a fluorescent product.

Alternatively, fluorescent compounds, such as fluorescein and rhodamine, may be 25 chemically coupled to antibodies without altering their binding capacity. When activated by illumination with light of a particular wavelength, the fluorochrome-labelled antibody adsorbs the light energy, inducing a state of excitability in the molecule, followed by emission of the light at a characteristic colour visually detectable with a light microscope. As in the EIA, the fluorescent labelled antibody is allowed to bind to the first antibody-hapten complex. After 30 washing off the unbound reagent, the remaining complex is then exposed to the light of the

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appropriate wavelength, the fluorescence observed indicates the presence of the hapten of interest. Immunofluorescence and EIA techniques are both very well established in the art and are particularly preferred for the present method. However, other reporter molecules, such as radioisotope, chemiluminescent or bioluminescent molecules, may also be employed. It will be readily apparent to the skilled technician how to vary the procedure to suit the required purpose.

The present invention is further described by reference to the following non-limiting Figures and Examples.

10

In the Figures:

Figure 1 is a schematic representation showing the nucleotide sequence of the exons and the intron/exon splice junctions of the ovine IL-5 gene [SEQ ID NO:1].

15

Figure 2 is a schematic representation showing the alignment of the deduced amino acid sequence of ovine IL-5 [SEQ ID NO:2] with human and mouse IL-5 polypeptides.

20

Figure 3 is a schematic representation showing an alignment of the deduced partial amino acid sequence of ovine IL-12 35kDa subunit [SEQ ID NO:6] with human and mouse IL-12 polypeptide molecules. The symbol (●) indicates that the amino acid is missing.

25

Figure 4 is a schematic representation showing the construction of the expression vector pCI-neo/p35, which expresses ovine IL-5 as a fusion protein with a polyhistidine (6xHis) polypeptide.

30

Figure 5 is a schematic representation showing the construction of a vector which expresses ovine IL-5 as a fusion protein with glutathione-S-transferase in a pGEX bacterial expression vector.

- 20 -

Figure 6 is a schematic representation showing the construction of a vector which expresses 35 kDa ovine IL-12 as a fusion protein with glutathione-S-transferase in a pGEX bacterial expression vector.

5 **Figure 7** is a schematic representation showing the construction of a vector which expresses 40 kDa ovine IL-12 as a fusion protein with glutathione-S-transferase in a pGEX bacterial expression vector.

10 **Figure 8** is a schematic representation showing the expression vector pCI-neo/IL-12 which co-expresses the 35 kDa and 40 kDa ovine IL-12 subunits under the control of the CMV I.E promoter/enhancer sequence.

15 **Figure 9** is a graphical representation showing the biological activity of recombinant ovine IL-5 (rOvIL-5) in a murine BAF cell (IL-5 dependent cell line) proliferation assay.

15

SEQ ID NOs referred to herein are summarised in Table 2.

20 Single and three letter abbreviations used for amino acid residues are shown in Table 3.

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TABLE 2
SEQUENCE IDENTITY NUMBERS

5	SEQ ID NO:	SEQUENCE
	1	Nucleotide sequence of exons from ovine IL-5 gene
	2	Amino acid sequence of ovine IL-5 derived from the
10		nucleotide sequence of the genomic clone
	3	Nucleotide sequence of ovine IL-5 cDNA
	4	Amino acid sequence of ovine IL-5 polypeptide derived from nucleotide sequence of cDNA clone
	5	Partial sequence of ovine IL-12 35kDa subunit cDNA
15	6	Partial amino acid sequence of ovine IL-12 35kDa subunit
	7	Nucleotide sequence of ovine IL-12 35kDa subunit cDNA
	8	Amino acid sequence of ovine IL-12 35kDA subunit
	9	Nucleotide sequence of ovine IL-12 40kDa subunit cDNA
	10	Amino acid sequence of ovine IL-12 40kDa subunit
20	11	Forward primer for cloning ovine IL-5 gene
	12	Reverse primer for cloning ovine IL-5 gene
	13	Forward primer for cloning ovine IL-5 cDNA
	14	Reverse primer for cloning ovine IL-5 cDNA
	15	Forward primer for cloning partial cDNA encoding ovine
25		IL-12 35kDa subunit
	16	Reverse primer for cloning partial cDNA encoding ovine
		IL-12 35kDa subunit
	17	Forward primer for cloning ovine IL-12 35kDa subunit cDNA
	18	Reverse primer for cloning ovine IL-12 35kDA subunit cDNA
30	19	Forward primer for cloning ovine IL-12 40kDA subunit cDNA
	20	Reverse primer for cloning ovine IL-12 40kDA subunit cDNA

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TABLE 3

Amino Acid	Three-letter Abbreviation	One-letter Symbol
Alanine	Ala	A
Arginine	Arg	R
Asparagine	Asn	N
Aspartic acid	Asp	D
5 Cysteine	Cys	C
Glutamine	Gln	Q
Glutamic acid	Glu	E
Glycine	Gly	G
Histidine	His	H
10 Isoleucine	Ile	I
Leucine	Leu	L
Lysine	Lys	K
Methionine	Met	M
Phenylalanine	Phe	F
20 Proline	Pro	P
Serine	Ser	S
Threonine	Thr	T
Tryptophan	Trp	W
Tyrosine	Tyr	Y
25 Valine	Val	V
Any residue	Xaa	X

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EXAMPLE 1
CLONING OF OVINE IL-5 GENE

5 An ovine genomic library constructed in EMBL3 vector was obtained from Clontech, USA. Phage were used to infect *Escherichia coli* strain K802. One hundred thousand plaques were transferred onto nylon filters and screened with a (³²P)-labelled fragment of human IL-5 cDNA. The filters were washed at 2xSSC, 0.1% (w/v) SDS, 65°C for 20 minutes. Five positive plaques were purified after three rounds of plating and screening. When the phage 10 DNA was used as the template for PCR, all five preparations of DNA gave a strong intense band of approximately 2 kb in size. The phage DNA was used as template in the PCR using the primers as follows:

CTT TCT TTG CCA AAG GCA AAC GC [SEQ ID No: 11] forward primer and
TGG CCC TCA TTC TCA CTG CA [SEQ ID No: 12] reverse primer.

15

The conditions for PCR were 30 cycles of 94°C for one min, 55°C for two min and 72°C for 2 min. The amplified PCR product was cloned into the *Sma*I site of pUC18 vector and 3 clones were sequenced by dideoxy sequencing using an automatic DNA sequencer.

20 One of the PCR products from clone 3-1 was chosen for cloning into pUC18 vector. The nucleotide sequence [SEQ ID NO: 1] of the exons of the ovine IL-5 gene is shown in Figure 1. Figure 2 shows the alignment of the deduced amino acid sequence [SEQ ID NO: 2] of ovine IL-5 with human and mouse IL-5. The overall amino acid homology of ovine IL-5 protein with human and mouse IL-5 molecules were 65% and 54%, respectively.

25

EXAMPLE 2
CLONING OF OVINE IL-5 cDNA

RNA from peripheral lymph node cells stimulated for 24 hours with Concanavalin 30 (5 μ g/ml) was isolated using Trizol (Gibco, BRL) according to the manufacturer's instructions.

- 24 -

RNA (5 µg) was reverse-transcribed to produce single-stranded cDNA, using Superscript RNase H-reverse transcriptase (Gibco- BRL). Ovine IL-5 cDNA sequences were then amplified in a polymerase chain reaction using Tag polymerase (Gibco- BRL) and the following primers:

CGCGGATCCATGCATCTGCGTTGACCTTG [SEQ ID No: 13] forward primer

TCAGCTTCCATGCTCCACTC [SEO ID No: 14] reverse primer.

The primers were based on the genomic sequence of ovine IL-5 gene set forth in SEQ ID NO: 1. The conditions for PCR were 30 cycles of amplification as follows:

10 94°C for 30 seconds;
55°C for 30 seconds; and
72°C for 30 seconds.

The amplified DNA was cloned into the *Sfi* site of pCRSCRIPTSK* (Stratagene, USA).
15 Four clones were sequenced in both directions using the M13 forward primer and the reverse primer using the Applied Biosystem 373A DNA sequencer. The complete nucleotide sequence of the IL-5 cDNA clone is set forth in SEQ ID NO: 3. The predicted amino acid sequence of full-length ovine IL-5 is set forth in SEQ ID NO: 4.

20

EXAMPLE 3

CLONING OVINE cDNA ENCODING PARTIAL 35-kDa SUBUNIT OF Π -12

1. Isolation and culture of ovine alveolar macrophages

25 A Merino lamb was euthanased and the lungs aseptically removed. The lungs were lavaged with 250 ml of phosphate buffered saline at pH7.3 containing 6 mM EDTA. Approximately 150 ml of this solution was then removed from the lungs via a sterile plastic tubing connected to a 50 ml syringe and the cells collected pelleted by centrifugation (500 g for 10 mins). The cells were washed twice in Dulbecco's modified Eagle's medium (Flow,
30 Australia) supplemented with 20 mM Hepes, 9 mM sodium bicarbonate, 2 mM glutamine, 50

- 25 -

μM 2-mercaptoethanol, 100 U/ml penicillin, 100 μg/ml streptomycin and 10% (v/v) heat-inactivated foetal bovine serum. The cells were resuspended in culture medium and viable cells enumerated by trypan blue exclusion. Cells were found to be greater than 95% macrophages as estimated by microscopic examination. The macrophages were cultured for 5 h at 37°C in 5_ 90 mm plastic tissue culture petri dishes (1×10^7 cells/dish) containing 12 ml of culture media and 20 μg/ml of lipopolysaccharide (Sigma, USA). The adhered macrophages were washed with PBS and then lysed in 1 ml Trizol (Gibco-BRL).

2. Reverse transcription - PCR

10 RNA from LPS-stimulate ovine alveolar macrophages lysed in Trizol were isolated according to manufacturer's instruction. An amount of 5 μg of RNA was used for first strand complementary DNA synthesis using Superscript RNase H- reverse transcriptase (Gibco-BRL) and PCR performed with Taq polymerase (Gibco-BRL) and the following primers:

15 and CGCGGATCCACCACCTCAGTTGCCAGG [SEQ ID No: 15] forward primer
CGCGGATCCGGCGTGAAGCAGGATGCAGAG [SEQ ID No: 16] reverse primer.

The amplified DNA fragment was subcloned into the BamHI site plasmid pUC18. DNA sequencing by the dideoxy termination method was performed on both strands using the 20 universal and reverse primers.

3. Cloning results

The nucleotide sequence of the partial cDNA encoding the 35 kDa subunit of IL-12 is set forth in SEQ ID NO: 5.

25

Figure 3 shows the alignment of the deduced partial amino acid sequence [SEQ ID NO: 6] of the 35 kDa subunit of ovine IL-12 compared to the bovine, human and mouse IL-12 cytokines. The level of amino acid homology with human and mouse equivalents are 79 and 61%, respectively.

30

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EXAMPLE 4

CLONING A FULL-LENGTH cDNA ENCODING THE 35 kDa SUBUNIT OF OVINE IL-12

5

Ovine alveolar macrophages were lipopolysaccharide (LPS)-stimulated as described in the preceding Examples for 4 hours. Macrophages were subsequently lysed in Trizol (Gibco BRL) and RNA was isolated according to the manufacturer's instructions. RNA (5 µg) was used as a template for first-strand cDNA synthesis using Superscript RNase H- reverse transcriptase 10 (Gibco-BRL). Ovine IL-12 sequences were amplified using the cDNA as a template and the following primers:

CGCCTCGAGATGTGCCGCCTCGCAGCCTC [SEQ ID No: 17] forward primer
and

CGCGGTACCCCTAGGAAGAACTCAGATAGCT [SEQ ID No: 18] reverse primer.

15

The amplified DNA fragment was subcloned into the *Sma*I site of plasmid pUC18. DNA sequencing was performed using the Applied Biosystem 373A DNA sequencer. Both strands were sequenced using the universal and reverse sequencing primers.

20 The nucleotide sequence of the full-length cDNA encoding the 35 kDa subunit of ovine IL-12 is set forth in SEQ ID NO: 7. The predicted amino acid sequence of the 35 kDa subunit of ovine IL-12 is set forth in SEQ ID NO: 8.

25

EXAMPLE 5

CLONING A FULL-LENGTH cDNA ENCODING THE 40 kDa SUBUNIT OF OVINE IL-12

Ovine peripheral lymph node cells were cultured with the phorbol ester, phorbol 30 myristate acetate (PMA) at 10 ng/ml and calcium ionophore A23187 (0.5 µg/ml) for 24 hours

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and the cells were lysed in TRizol (Gibco BRL) according to the manufacturer's instructions. RNA was isolated and PCR performed with the following primers:

CGCGGATCCATGCACCCTCAGCAGTTGGTC [SEQ ID NO: 19] forward primer
and

5 CGCGTCGACACTGCAGGACACAGATGCCA [SEQ ID No: 20] reverse primer.

The PCR product was cloned into the *Sma*I site of the plasmid pUC18 and four clones were sequenced with the M13 universal and reverse sequencing primers using the Applied Biosystem 373A DNA sequencer. The sequencing reactions were performed using the PRISM™
10 Dye Deoxy™ Terminator Cycle Sequencing Kit (Applied Biosystems). Using these primers approximately 350–400 nucleotide sequence could be determined from the 5' and 3' ends of the cDNA.

1. Subcloning step

15 One of the full-length cDNAs encoding the 40 kDa subunit of ovine IL-12 was digested with *Sau*3AI and *Eco*RI and a DNA fragment of approximately 400bp was subcloned into the *Bam*HI/*Eco*RI site of pUC118. This subclone was sequenced using the universal and reverse primers as described above.

20 The complete nucleotide sequence of the full-length cDNA encoding the 40 kDa subunit of ovine IL-12 is set forth in SEQ ID NO: 9. The predicted amino acid sequence of the full-length 40 kDa subunit of ovine IL-12 is set forth in SEQ ID NO: 10.

25

EXAMPLE 6

IL-5 BIOASSAY USING BAF MOUSE CELL LINE

IL-5 dependent murine BAF cells were grown in the presence of murine X63 cell
30 line supernatant [5%(v/v)] as an IL-5 source, in DMEM /10%(w/v)FCS. A well-grown cell

culture, grown in a 75cm³ flask was decanted into a 50 ml centrifuge tube and centrifuged at 1200 rpm for 10 mins. Cells were then resuspended in 10 % (v/v) DMEM and recentrifuged at 1200rpm for 10 mins. Cell washes were repeated twice to remove exogenous IL-5 and finally resuspended to a concentration of 5 X 10⁴ cells /ml. Recombinant ovine IL-5 protein 5 generated from the expression system was titrated in triplicate across a 96 well tissue culture plate then 100 µL of the washed BAF cell suspension was added to a final concentration of 5 X 10³ cells/well . Murine IL-5 was used as a positive control for cell proliferation. The cell cultures were incubated in 5%(v/v) CO₂ at 37°C for 2 days then pulsed for 8-18 hr with tritiated thymidine, harvested and counted to determine the amount of radioactivity
10 incorporated.

EXAMPLE 7 CYTOKINES

15

Recombinant ovine IL-5 and IL-12 are prepared basically as described for the preparation of recombinant ovine IL-1 β in International Patent Application No: PCT/AU91/00419. Briefly, IL-5 and IL-12 are prepared as follows:

20 1. Construction of ovine interleukin-5 expression vector

The ovine IL-5 cDNA encoding the mature IL-5 protein was obtained as described in Example 2.

In one embodiment, the coding sequence for the mature form of the IL-5 gene was 25 PCR-amplified using a thermostable polymerase and introduced into the polylinker of an expression vector belonging to the pQE-30 series of vectors, wherein the vector was selected such that the reading frame of IL-5 mature form coding sequence was in-frame with the reading frame of polyhistidine (6xHis) contained therein. The resultant expression construct was designated pQE-30-IL-5. Figure 4 is a schematic illustration of the
30 expression construct pQE-30-IL-5. The pQE-30-IL-5 expression construct was introduced

into *E.coli* strain DH5 α , where induction of the P5 promoter results in high level expression of a fusion protein comprising the polyHis and IL-5 polypeptides.

In an alternative embodiment, the cDNA insert is ligated into the multiple cloning site of an expression vector belonging to the pGEX series of vectors, wherein the vector is selected such that the reading frame of IL-5 is in-frame with the reading frame of glutathione-s-transferase contained therein (Smith and Johnson, 1988). The IL-5 open reading frame is cloned immediately downstream of the thrombin cleavage site, to produce an in-frame fusion. The recombinant plasmid is designated pGEX-IL-5. Figure 5 is a schematic illustration of the expression plasmid pGEX-IL-5 showing the site of thrombin cleavage of the fusion protein. Transformants of *Escherichia coli* strain JM109 are then produced.

2. Expression and affinity purification of recombinant interleukin-5

15

To express IL-5 under the control of the P5 promoter, bacterial colonies transformed with pQE-30-IL-5 (see above, Example 7.1) were picked and cultured overnight at 37°C in LB growth medium [1%(w/v) tryptone, 0.5%(w/v) yeast extract, 1% (w/v) NaCl] supplemented with ampicillin (50 μ g/ml). Flasks containing 1L of LB growth medium and 20 ampicillin (50 μ g/ml) and a 1:50 inoculum of overnight cultures were shaken at 37°C. After 2 hours, the P5 promoter of the expression construct pQE-30-IL-5 was induced with IPTG to a final concentration of 1mM and incubated for a further for two hours.

To purify the recombinant IL-5 protein, 2ml of a 50% slurry of Ni-NTA resin 25 (Clontech) were first equilibrated with PBS. The bacterial cells expressing the polyhistidine-IL-5 fusion protein were recovered by centrifugation at 4000 g for 10 min and the pellet sonicated in 2.5% (v/v) Zwittergent (Sigma, product No T7763). The sonicate was mixed with the Ni-NTA slurry for 30min. Unbound proteins were removed from the supernatant fraction following centrifugation at 800 g. Recombinant IL-5 was eluted from 30 the Ni-NTA slurry with 1 bed volume of 50mM imidazole. Multiple eluants were collected

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to maximize yield.

When IL-5 is expressed under the control of the *tac* promoter, overnight cultures of the pGEX-IL-5 plasmid are diluted in 250ml of Luria Broth (10g/L bacto-tryptone, 5g/L yeast extract, 10g/L NaCl) or Terrific Broth (16.43g/L K₂HPO₄.3H₂O, 2.31g/L KH₂PO₄, 5g/L tryptone 12g/L, yeast 24g/L, glycerol 4ml/L) containing 100/ μ g/ml ampicillin. The cultures are grown for 2h at 37°C before adding IPTG (isopropyl- β -thiogalactopyranoside) to 0.2mM (or as indicated in the legend). Induction of the *tac* promoter results in high-level expression of a fusion protein between GST and IL-5. After 4h, the cultures are harvested 10 and centrifuged. The pellets are weighed and resuspended in the appropriate volume of buffer (50mM Tris/HCl, pH7.5; 10ml/g of wet weight of pellet). The cells are lysed on ice by sonication and then centrifuged.

To purify the recombinant GST-IL-5 fusion protein, the supernatant is loaded onto a 15 5ml glutathione Sepharose column (sulphur-linkage, Sigma). The flow through is retained and the column is then washed thoroughly with at least 5 bed volumes of 50mM Tris/HCl, pH7.5. The recombinant IL-5 protein is eluted either as a fusion product with 5mM glutathione or as free IL-5 by cleavage with human thrombin (10U/ml; ICN) at room temperature for 1h. The eluted proteins are analysed by electrophoresis on a 15% (w/v) 20 SDS/polyacrylamide gel and visualised by staining with 0.05% (w/v) Coomassie Brilliant Blue R.

3. Expression and purification of recombinant ovine IL-12 polypeptides

The cDNAs encoding the mature form of the 35 kDa and 40 kDa subunits of ovine 25 IL-1 β are cloned into the multiple cloning site of an expression vector belonging to the pGEX series of vectors, wherein the vector is selected such that the reading frame of each IL-12 cDNA sequence is in-frame with the reading frame of glutathione-s-transferase contained therein (Smith and Johnson, 1988), to produce the expression plasmids pGEX-IL12a and pGEX-IL12b, respectively. Figures 6 and 7 are schematic illustrations of the 30 expression plasmids pGEX-IL12a and pGEX-IL12b respectively, showing the sites of

thrombin cleavage of the fusion protein. Induction of the tac promoter of the expression plasmids pGEX-IL12a and pGEX-IL12b results in high level expression of a fusion protein in each case.

5 Affinity chromatography of the fusion proteins on a glutathione-Sepharose column, followed by cleavage with thrombin, yields the free form of the mature IL-12 35 kDa and 40 kDa subunits. Alternatively, elution from the column with glutathione yields GST-IL12 fusion proteins with approximate molecular weights of 61kDa for the 35 kDa IL-12 subunit) and 66 kDa (for the 40 kDa IL-12 subunit).

10

4. Expression of recombinant ovine IL-12 heterodimer

The cDNA encoding the 35kDa subunit of ovine IL-12 is cloned into the multiple cloning site of the mammalian expression vector pCI-neo, between the CMV I.E 15 enhancer/promoter/ intron sequence and the SV40 late polyadenylation sequence, to produce the intermediate expression vector pCI-neo/p35. Expression of the 35 kDa IL-12 polypeptide in pCI-neo/p35 is under the control of the CMV I.E enhancer/promoter and chimeric intron sequences

20 The cDNA encoding the 40kDa subunit of ovine IL-12 is cloned into the multiple cloning site of the mammalian expression vector pSI, between the CMV I.E enhancer/promoter/intron and the SV40 late polyadenylation sequence, to produce the intermediate vector pSI/p40. Expression of the 40 kDa IL-12 polypeptide in pSI/p40 is under the control of the CMV I.E enhancer/promoter and chimeric intron sequences.

25

An expression cassette comprising the 40 kDa IL-12 subunit coding sequence, together with the CMV I.E. enhancer/promoter, chimeric intron and SV40 polyadenylation sequence is removed from the pSI/p40 construct and introduced into a compatible site in the pCI-neo/p35 vector construct to create a dual construct, designated pCI-neo/IL-12, that 30 contains open reading frames encoding both the 35 kDa and 40 kDa polypeptides in their

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respective expression cassettes and under the operable control of the CMV I.E promoter/enhancer sequences. Figure 8 is a schematic representation of the dual expression construct pCI-neo/IL-12.

5 The construct pCI-neo/IL-12 is introduced into a mammalian cell line for transient or stable expression. Expression is constitutive for both genes. The IL-12 heterodimer forms from the subunits and is secreted into the culture medium.

5. Protein assays

10 Protein concentrations are estimated by the Bradford dye assay (Biorad) using bovine serum albumin as standard.

EXAMPLE 8

15

BIOASSAY OF RECOMBINANT IL-5

Recombinant IL-5 (rOvIL-5) was prepared and purified from cells transformed with the expression construct pQE-30-IL-5 as described in Example 7 and subsequently assayed for 20 biological activity using the BAF mouse cell line bioassay described in Example 6. As shown in Figure 9, significant biological activity above that observed for control samples, was detected for rOvIL-5 produced using the expression vector pQE-30-IL-5 (Figure 4), when the recombinant polypeptide was present in the assay samples at a concentration greater than 1/32 dilution.

25

EXAMPLE 9 VACCINE PREPARATIONS

30 The recombinant *Taenia ovis* fusion protein GST-45W (Johnson et al., 1989) is used

as the model antigen in all studies. Vaccines are formulated with 50 μ g/dose of GST-45W and 0, 10 or 100 μ g/dose of recombinant IL-5 and/or 35 kDa IL-12 and/or 40 kDa IL-12 polypeptides in either phosphate buffered saline (PBS) or the conventional adjuvants Quil A (1 or 5mg/ml), incomplete Freund's adjuvant (IFA; 1:1, oil:water) and aluminium hydroxide 5 (6 mg/ml). Sheep are injected intramuscularly (i/m) (1ml) into the left hind leg for the primary inoculation and 4 weeks later boosted with an i/m injection of the same vaccine preparation into the right hind leg.

10

EXAMPLE 10
SEROLOGY

Sera are collected from all animals before the primary inoculations and then at weekly intervals until 4 weeks post secondary inoculation. Sera are stored at -20°C until 15 assayed for antibodies to 45W using the enzyme immunoassay (EIA) described below. Pre-bleed sera from all sheep are screened for antibodies to 45W prior to the commencement of experiments and any animals demonstrating significant antibody levels to 45W (EIA OD > 0.2 at 1/300 serum dilution) were excluded. For the EIA, either recombinant 45W, thrombin cleaved and purified from the GST moiety or GST-45W (as indicated), is bound to 20 96-well microtitre plates (Nunc Maxisorb) by incubating 0.2 μ g per well in 100 μ l of 50mM carbonate buffer (pH 9.6) for 20hrs at 20°C. The plates are then post-coated (1hr at 20°C) with 100 μ l per well of phosphate buffered saline (PBS: 0.9% w/v, pH 7.2) containing 1% (w/v) sodium casein. After 4 washes with phosphate buffered saline containing 0.05% v/v Tween 20 (PBST), 100 μ l of serial dilutions of serum samples are added to the wells for 1hr 25 at 20°C. The plates are then washed 4 times with PBST before the addition of 100 μ l per well of a 1/1000 dilution of horseradish peroxidase conjugated anti-ovine IgG monoclonal antibody (VET05, Silenus, Australia) in PBST for 1 hr at 20°C. Plates are washed 5 times with PBST and 100 μ l of tetra-methyl benzidine (TMB) substrate (Bos et al, 1981) added to each well for 30min at 20°C before the reaction is stopped by the addition of 50 μ l of 0.5M 30 H₂SO₄ per well and the absorbance read at 450nm.

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EXAMPLE 11
ADJUVANT ACTIVITY OF RECOMBINANT IL-5

5 Sheep are randomly allocated into 12 groups of 5 animals. Serum samples were collected prior to first vaccination and then at weekly intervals until 4 weeks post secondary vaccination. Serum antibody levels to purified 45W are quantified by EIA. All vaccine formulations are standardised to contain 50µg of GST-45W per dose. The addition of at least 10-100µg of recombinant IL-5 to aqueous and aluminium hydroxide (AlOH) vaccine 10 formulations results in significant increases in serum IgG anti-45W.

EXAMPLE 12
ADJUVANT ACTIVITY OF RECOMBINANT IL-12

15 An experiment similar to that described in Example 11 for recombinant IL-5, is conducted for the co-expressed recombinant IL-12 35 kDa and 40 kDa polypeptide subunits, produced from the plasmid pCI-neo/IL-12 (Example 7.4), to ascertain the adjuvant potential of recombinant IL-12. Serum antibody levels are quantified by EIA using GST-45W antigen. As in Example 11, adjuvant effects are seen in the PBS and AlOH vaccine groups 20 when approximately at least 10-100µg of recombinant IL-12 is incorporated.

EXAMPLE 13
**ADJUVANT ACTIVITY OF RECOMBINANT IL-5 AND
IL-12 IN COMBINATION**

25 The combination of both IL-5 and IL-12 is studied in AlOH vaccine formulations. Table 4 shows the vaccine formulations used for this experiment. Animals (five per group) are injected i/m in the rear leg on day 0 and receive a second i/m injection in the opposing 30 rear leg on day 28. The cytokines exert synergistic co-adjuvant effects when administered

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with AIOH. Antibody titres are elevated significantly compared to titres obtained when AIOH alone is used as adjuvant. The level of antibody obtained with the AIOH-cytokine combination is commensurate with that obtained with Quil A.

TABLE 4

Vaccine formulations comprising combinations of recombinant
IL-5 and IL-12 polypeptides

VACCINE FORMULATION	μg of recombinant ovine interleukin	
	IL-5	IL-12
1. AIOH	0	0
2. AIOH	10-100	0
3. AIOH	0	10-100
4. AIOH	10-100	10-100
5. QUILA	0	
6. Controls (no vaccine)	0	

Those skilled in the art will appreciate that the invention described herein is susceptible to variations and modifications other than those specifically described. It is to be understood that the invention includes all such variations and modifications. The invention also includes all of the steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any and all combinations of any two or more of said steps or features.

REFERENCES:

1. BALKWILL F.R. and BURKE F. *Immunol. Today* 9: 299, 1989.
2. BIELEFELD T., OHMANN H., LAWMAN M.J.P. and BABIUK L.A. *Antiviral Res.* 7: 187, 1987.
3. BOS E.S., van der DOELEN A.A., van ROOY N. and SCHUURS A.H.W.M. J. *Immunoassay* 2: 187-204, 1981.
4. GEARING A.J.H., BIRD C.R., BRISTOW A., POOLE S. and THORPE R. J. *Immunol.* 99: 7-11, 1987.
5. JOHNSON K.S., HARRISON G.B.L., LIGHTOWLERS M.W., P'HOY K.L., COUGLE W.G., DEMPSTER R.P., LAWRENCE S.B., VINTON J.G., HEATH D.D. and RICKARD M.D. *Nature* 338: 585-587, 1989.
6. KOBAYASHI *et al* *J. Exp. Med.* 70: 827, 1989.
7. MANIATIS T., FRITSCH E.F. and SAMBROOK J. In: Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor N.Y., 1982.
8. METCALF D. *Immunol. Cell. Biol.* 65: 35, 1987.
9. SAMBROOK *et al*. In: Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor N.Y., 1989.
10. SMITH D.B. and JOHNSON K.S. *Gene* 67: 31-40, 1988.

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11. SONADA *et al.* *Cellular Immunology*, 158: 172, 1992.
12. STERN *et al.* *Proc. Natl. Acad. Sci.* 87: 6808, 1990.

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: COMMONWEALTH SCIENTIFIC AND INDUSTRIAL RESEARCH ORGANISATION

(ii) TITLE OF INVENTION: IMMUNE RESPONSE MODULATORS AND USES THEREFOR

(iii) NUMBER OF SEQUENCES: 20

(iv) CORRESPONDENCE ADDRESS:

(A) ADDRESSEE: DAVIES COLLISON CAVE
(B) STREET: 1 LITTLE COLLINS STREET
(C) CITY: MELBOURNE
(D) STATE: VICTORIA
(E) COUNTRY: AUSTRALIA
(F) ZIP: 3000

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER: PCT INTERNATIONAL
(B) FILING DATE: 14-JUN-1996

(vii) PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER: AU PN3502/95
(B) FILING DATE: 14-JUN-1995

(A) APPLICATION NUMBER: AU PN6244/95
(B) FILING DATE: 27-OCT-1995

(viii) ATTORNEY/AGENT INFORMATION:

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(ix) TELECOMMUNICATION INFORMATION:

(A) TELEPHONE: +61 3 9254 2777
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(2) INFORMATION FOR SEO ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 520 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:

- (A) NAME/KEY: CDS
(B) LOCATION: 46 441

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1

CTTTCTTG CAAAGGCAAA CGCTGAACAT TTCAAGAGTC AGAGA ATG CAT CTG 54

Mat. Hist. Inv.

1

```

CGT TTG ACC TTG GTA GCT CTT GGA GCT GCC TAT GTT TGT GCC AAT GCT 102
Arg Leu Thr Leu Val Ala Leu Gly Ala Ala Tyr Val Cys Ala Asn Ala
          5       10      15

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GTA GAA AGT ACC ATG AAT AGA CTG GTG GCA GAG ACC TTG ACA CTG CTC 150
Val Glu Ser Thr Met Asn Arg Leu Val Ala Glu Thr Leu Thr Leu Leu
   20          25          30          35

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TCC ACG CAT CAA ACT CTG CTG ATA GGT GAT GGG AAC TTG ATG ATT CCT 198
 Ser Thr His Gln Thr Leu Leu Ile Gly Asp Gly Asn Leu Met Ile Pro
 40 45 50

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ACT CCT CAG CAT ACA AAT CAC CAA CTA TGC ATT GAA GAA GTC TTT CAG 246
Thr Pro Gln His Thr Asn His Gln Leu Cys Ile Glu Glu Val Phe Gln
          55           60           65

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GGA ATA GAC ACA TTG AAG AAT CAA ACT GCA CAA GGG GAT GCT GTG AAA
 Gly Ile Asp Thr Leu Lys Asn Gln Thr Ala Gln Gly Asp Ala Val Lys
 70 75 80

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AAA ATA TTC CGA AAC TTG TCT TTA ATA AAA GAA TAC ATA GAC CTC CAA 342
Lys Ile Phe Arg Asn Leu Ser Leu Ile Lys Glu Tyr Ile Asp Leu Gln
          85           90           95

```

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AAA AGG AAG TGT GGA GGA GAA AGA TGG AGA GTG AAA CAA TTC CTC GAC	390		
Lys Arg Lys Cys Gly Gly Glu Arg Trp Arg Val Lys Gln Phe Leu Asp			
100	105	110	115
TAC CTG CAA GTT TTC CTT GGT GTG ATA AAC ACA GAG TGG ACG ATG GAA	438		
Tyr Leu Gln Val Phe Leu Gly Val Ile Asn Thr Glu Trp Thr Met Glu			
120	125	130	
AGC TGA GAT CTA CCT CTC TCA CTG TAG TGA AAG TTT CTG GAG GAG GAG	486		
Ser *			
AAG GAT GTT TTA ATT GCA GTC AGA ATG AGG GCC A	520		

(3) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 132 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met His Leu Arg Leu Thr Leu Val Ala Leu Gly Ala Ala Tyr Val Cys			
1	5	10	15
Ala Asn Ala Val Glu Ser Thr Met Asn Arg Leu Val Ala Glu Thr Leu			
20	25	30	
Thr Leu Leu Ser Thr His Gln Thr Leu Leu Ile Gly Asp Gly Asn Leu			
35	40	45	
Met Ile Pro Thr Pro Gln His Thr Asn His Gln Leu Cys Ile Glu Glu			
50	55	60	
Val Phe Gln Gly Ile Asp Thr Leu Lys Asn Gln Thr Ala Gln Gly Asp			
65	70	75	80
Ala Val Lys Lys Ile Phe Arg Asn Leu Ser Leu Ile Lys Glu Tyr Ile			
85	90	95	

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Asp Leu Gln Lys Arg Lys Cys Gly Gly Glu Arg Trp Arg Val Lys Gln
100 105 110

Phe Leu Asp Tyr Leu Gln Val Phe Leu Gly Val Ile Asn Thr Glu Trp
115 120 125

Thr Met Glu Ser *

(4) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 399 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

- (A) NAME/KEY: CDS
(B) LOCATION: 1 396

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```

ATG CAT CTG CGT TTG ACC TTG GTA GCT CTT GGA GCT GCC TAT GTT TGT 48
Met His Leu Arg Leu Thr Leu Val Ala Leu Gly Ala Ala Tyr Val Cys
   1           5           10          15

```

```

GCC AAT GCT GTA GAA AGT ACC ATG AAT AGA CTG GTG GCA GAG ACC TTG 96
Ala Asn Ala Val Glu Ser Thr Met Asn Arg Leu Val Ala Glu Thr Leu
          20      25      30

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ACA CTG CTC TCC ACG CAT CAA ACT CTG CTG ATA GGT GAT GGG AAC TTG 144
Thr Leu Leu Ser Thr His Gln Thr Leu Leu Ile Gly Asp Gly Asn Leu
          35           40           45

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ATG ATT CCT ACT CCT CAG CAT ACA AAT CAC CAA CTA TGC ATT GAA GAA 192
Met Ile Pro Thr Pro Gln His Thr Asn His Gln Leu Cys Ile Glu Glu
          50      55      60

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GTC TTT CAG GGA ATA GAC ACA TTG AAG AAT CAA ACT GCA CAA GGG GAT	240		
Val Phe Gln Gly Ile Asp Thr Leu Lys Asn Gln Thr Ala Gln Gly Asp			
65	70	75	80
 GCT GTG AAA AAA ATA TTC CGA AAC TTG TCT TTA ATA AAA GAA TAC ATA	288		
Ala Val Lys Lys Ile Phe Arg Asn Leu Ser Leu Ile Lys Glu Tyr Ile			
85	90	95	
 GAC CTC CAA AAA AGG AAG TGT GGA GGA GAA AGA TGG AGA GTG AAA CAA	336		
Asp Leu Gln Lys Arg Lys Cys Gly Gly Glu Arg Trp Arg Val Lys Gln			
100	105	110	
 TTC CTC GAC TAC CTG CAA GTT TTC CTT GGT GTG ATA AAC ACA GAG TGG	384		
Phe Leu Asp Tyr Leu Gln Val Phe Leu Gly Val Ile Asn Thr Glu Trp			
115	120	125	
 ACG ATG GAA AGC TGA	399		
Thr Met Glu Ser			
130			

(5) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 132 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met His Leu Arg Leu Thr Leu Val Ala Leu Gly Ala Ala Tyr Val Cys			
1	5	10	15

Ala Asn Ala Val Glu Ser Thr Met Asn Arg Leu Val Ala Glu Thr Leu			
20	25	30	

Thr Leu Leu Ser Thr His Gln Thr Leu Leu Ile Gly Asp Gly Asn Leu			
35	40	45	

Met Ile Pro Thr Pro Gln His Thr Asn His Gln Leu Cys Ile Glu Glu			
50	55	60	

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Val Phe Gln Gly Ile Asp Thr Leu Lys Asn Gln Thr Ala Gln Gly Asp
65 70 75 80

Ala Val Lys Lys Ile Phe Arg Asn Leu Ser Leu Ile Lys Glu Tyr Ile
85 90 95

Asp Leu Gln Lys Arg Lys Cys Gly Gly Glu Arg Trp Arg Val Lys Gln
100 105 110

Phe Leu Asp Tyr Leu Gln Val Phe Leu Gly Val Ile Asn Thr Glu Trp
115 120 125

Thr Met Glu Ser
130

(6) INFORMATION FOR SEQ ID NO:5:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 554 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA (partial)

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 3..554

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

AC CAC CAC CTC AGT TTG GCC AGG AGC CTG CCC ACC ACC ACA GCA GGC	47
His His Leu Ser Leu Ala Arg Ser Leu Pro Thr Thr Thr Ala Gly	
1 5 10 15	

CCA GGA AGG AGT TGC CTT GAC TAC TCC CAA AAC ACC CTG CTG AGG GCC GTC	95
Pro Gly Arg Ser Cys Leu Asp Tyr Ser Gln Asn Leu Leu Arg Ala Val	
20 25 30	

AGC AAC ACG CTG CAG AAG GCC AGA CAA ACC CTA GAA TTT TAC TCC TGC	143
Ser Asn Thr Leu Gln Lys Ala Arg Gln Thr Leu Glu Phe Tyr Ser Cys	
35 40 45	

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ACT TCT GAG GAG ATT GAT CAT GAA GAT ATC ACC AAA GAT AAA ACC AGC	191		
Thr Ser Glu Glu Ile Asp His Glu Asp Ile Thr Lys Asp Lys Thr Ser			
50	55	60	
ACA GTG GAG GCC TGT TTA CCA CTG GAA TTA GCC ACG AAT GAG AGT TGT	239		
Thr Val Glu Ala Cys Leu Pro Leu Glu Leu Ala Thr Asn Glu Ser Cys			
65	70	75	
CTG GCT TCC AGA GAG ACC TCT TTA ATA ACT AAT GGG CAT TGT CTG TCT	287		
Leu Ala Ser Arg Glu Thr Ser Leu Ile Thr Asn Gly His Cys Leu Ser			
80	85	90	95
TCT GGA AAG ACC TCT TTT ATG ACA ACC CTG TGC CTT AGA AGT ATC TAC	335		
Ser Gly Lys Thr Ser Phe Met Thr Thr Leu Cys Leu Arg Ser Ile Tyr			
100	105	110	
AAG GAC TTG AAG ATG TAT CAC ATG GAG TTC CAG GCC ATG AAT GCA AAG	383		
Lys Asp Leu Lys Met Tyr His Met Glu Phe Gln Ala Met Asn Ala Lys			
115	120	125	
CTT CTG ATG GAT CCT AAG AGG CAA GTC TTT CTA GAC CAG AAC ATG CTG	431		
Leu Leu Met Asp Pro Lys Arg Gln Val Phe Leu Asp Gln Asn Met Leu			
130	135	140	
GCA GCT ATT GCT GAG CTA ATG CAG GCC CTG AAT TTC GAC AGT GAG ACT	479		
Ala Ala Ile Ala Glu Leu Met Gln Ala Leu Asn Phe Asp Ser Glu Thr			
145	150	155	
GTG CCA CAG AAA CCC TCC CTG GAA GAA CTG GAT TTT TAT AAG ACA AAA	527		
Val Pro Gln Lys Pro Ser Leu Glu Glu Leu Asp Phe Tyr Lys Thr Lys			
160	165	170	175
GTC AAG CTC TGC ATC CTG CTT CAC GCC	554		
Val Lys Leu Cys Ile Leu Leu His Ala			
180			

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(7) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 184 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

His His Leu Ser Leu Ala Arg Ser Leu Pro Thr Thr Thr Ala Gly Pro
1 5 10 15

Gly Arg Ser Cys Leu Asp Tyr Ser Gln Asn Leu Leu Arg Ala Val Ser
20 25 30

Asn Thr Leu Gln Lys Ala Arg Gln Thr Leu Glu Phe Tyr Ser Cys Thr
35 40 45

Ser Glu Glu Ile Asp His Glu Asp Ile Thr Lys Asp Lys Thr Ser Thr
50 55 60

Val Glu Ala Cys Leu Pro Leu Glu Leu Ala Thr Asn Glu Ser Cys Leu
65 70 75 80

Ala Ser Arg Glu Thr Ser Leu Ile Thr Asn Gly His Cys Leu Ser Ser
85 90 95

Gly Lys Thr Ser Phe Met Thr Thr Leu Cys Leu Arg Ser Ile Tyr Lys
100 105 110

Asp Leu Lys Met Tyr His Met Glu Phe Gln Ala Met Asn Ala Lys Leu
115 120 125

Leu Met Asp Pro Lys Arg Gln Val Phe Leu Asp Gln Asn Met Leu Ala
130 135 140

Ala Ile Ala Glu Leu Met Gln Ala Leu Asn Phe Asp Ser Glu Thr Val
145 150 155 160

Pro Gln Lys Pro Ser Leu Glu Glu Leu Asp Phe Tyr Lys Thr Lys Val
165 170 175

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Lys Leu Cys Ile Leu Leu His Ala
180

(8) INFORMATION FOR SEO ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 666 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(iii) MOLECULE TYPE: cDNA

(ix) FEATURE:

- (A) NAME/KEY: CDS
(B) LOCATION: 1 663

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

ATG TGC CCG CTT CGC AGC CTC CTC CTC ATA TCC ACC CTG GTT CTC CTC	48
Met Cys Pro Leu Arg Ser Leu Leu Leu Ile Ser Thr Leu Val Leu Leu	
1 5 10 15	
CAC CAC CTG CCC CAC CTC AGT TTG GGC AGG AGC CTG CCC ACC ACC ACA	96
His His Leu Pro His Leu Ser Leu Gly Arg Ser Leu Pro Thr Thr Thr	
20 25 30	
GCA GGC CCA GGA ACG AGT TGC CTT GAC TAC TCC CAA AAC CTG CTG AGG	14
Ala Gly Pro Gly Thr Ser Cys Leu Asp Tyr Ser Gln Asn Leu Leu Arg	
35 40 45	
GCC GTC AGC AAC ACG CTG CAG AAG GCC AGA CAA ACC CTA GAA TTT TAC	19
Ala Val Ser Asn Thr Leu Gln Lys Ala Arg Gln Thr Leu Glu Phe Tyr	
50 55 60	
TCC TGC ACT TCT GAG GAG ATT GAT CAT GAA GAT TTA ACC AAA GAT AAA	24
Ser Cys Thr Ser Glu Glu Ile Asp His Glu Asp Leu Thr Lys Asp Lys	
65 70 75 80	
ACC AGC ACA GTG GAG GGC TGT TTA CCA CTG GAA TTA GCC ACG AAT GAG	29
Thr Ser Thr Val Glu Gly Cys Leu Pro Leu Glu Leu Ala Thr Asn Glu	
85 90 95	

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AGT TGT CTG GCT TCC AGA GAG ACC TCT TTA ATA ACT AAT GGG CAT TGT	336
Ser Cys Leu Ala Ser Arg Glu Thr Ser Leu Ile Thr Asn Gly His Cys	
100	105
	110
CTG TCT CCT GGA AAG ACT TCT TTT ATG ACA ACC CTG TGC CTT AGA AGT	384
Leu Ser Pro Gly Lys Thr Ser Phe Met Thr Thr Leu Cys Leu Arg Ser	
115	120
	125
ATC TAC AAG GAC TTG AAG ATG TAT CAC ATG GAG TTC CAG GCC ATG AAT	432
Ile Tyr Lys Asp Leu Lys Met Tyr His Met Glu Phe Gln Ala Met Asn	
130	135
	140
GCA AAG CTT CTG ATG GAT CCT AAG AGG CAA GTC TTT CTA GAC CAG AAC	480
Ala Lys Leu Leu Met Asp Pro Lys Arg Gln Val Phe Leu Asp Gln Asn	
145	150
	155
ATG CTG GCA GCT ATT GCT GAG CTA ATG CAG GCC CTG AAT TTC GAC AGT	528
Met Leu Ala Ala Ile Ala Glu Leu Met Gln Ala Leu Asn Phe Asp Ser	
165	170
	175
GAG ACT GTG CCA CAG AAA CCC TCC CTG GAA GAA CTG GAT TTT TAT AAG	576
Glu Thr Val Pro Gln Lys Pro Ser Leu Glu Glu Leu Asp Phe Tyr Lys	
180	185
	190
ACA AAA ATC AAG CTC TGC ATC CTT CTT CAC GCC TTC AGA ATT CGT GCG	624
Thr Lys Ile Lys Leu Cys Ile Leu Leu His Ala Phe Arg Ile Arg Ala	
195	200
	205
G TG ACC ATC GAC AGA ATG ATG AGC TAT CTG AGT TCT TCC TAG	666
Val Thr Ile Asp Arg Met Met Ser Tyr Leu Ser Ser Ser	
210	215
	220

(9) INFORMATION FOR SEQ ID NO:8:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 221 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Met Cys Pro Leu Arg Ser Leu Leu Ile Ser Thr Leu Val Leu Leu			
1	5	10	15

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His His Leu Pro His Leu Ser Leu Gly Arg Ser Leu Pro Thr Thr Thr
20 25 30

Ala Gly Pro Gly Thr Ser Cys Leu Asp Tyr Ser Gln Asn Leu Leu Arg
35 40 45

Ala Val Ser Asn Thr Leu Gln Lys Ala Arg Gln Thr Leu Glu Phe Tyr
50 55 60

Ser Cys Thr Ser Glu Glu Ile Asp His Glu Asp Leu Thr Lys Asp Lys
65 70 75 80

Thr Ser Thr Val Glu Gly Cys Leu Pro Leu Glu Leu Ala Thr Asn Glu
85 90 95

Ser Cys Leu Ala Ser Arg Glu Thr Ser Leu Ile Thr Asn Gly His Cys
100 105 110

Leu Ser Pro Gly Lys Thr Ser Phe Met Thr Thr Leu Cys Leu Arg Ser
115 120 125

Ile Tyr Lys Asp Leu Lys Met Tyr His Met Glu Phe Gln Ala Met Asn
130 135 140

Ala Lys Leu Leu Met Asp Pro Lys Arg Gln Val Phe Leu Asp Gln Asn
145 150 155 160

Met Leu Ala Ala Ile Ala Glu Leu Met Gln Ala Leu Asn Phe Asp Ser
165 170 175

Glu Thr Val Pro Gln Lys Pro Ser Leu Glu Glu Leu Asp Phe Tyr Lys
180 185 190

Thr Lys Ile Lys Leu Cys Ile Leu Leu His Ala Phe Arg Ile Arg Ala
195 200 205

Val Thr Ile Asp Arg Met Met Ser Tyr Leu Ser Ser Ser
210 215 220

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(10) INFORMATION FOR SEQ ID NO:9:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 984 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..981

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

ATG CAC CCT CAG CAG TTG GTC GTT TCC TGG TTT TCC CTG GTT TTG CTG	48
Met His Pro Gln Gln Leu Val Val Ser Trp Phe Ser Leu Val Leu Leu	
1 5 10 15	
GCA TCT CCC ATC GTG GCC ATA TGG GAA CTG GAG AAA AAT GTT TAT GTT	96
Ala Ser Pro Ile Val Ala Ile Trp Glu Leu Glu Lys Asn Val Tyr Val	
20 25 30	
GTA GAA TTG GAT TGG TAT CCT AAT GCT CCT GGA GAA ACA GTG GTC CTC	144
Val Glu Leu Asp Trp Tyr Pro Asn Ala Pro Gly Glu Thr Val Val Leu	
35 40 45	
ACA TGT GAC ACT CCT GAA GAA GAT GGC ATC ACC TGG ACC TCA GAC CAG	192
Thr Cys Asp Thr Pro Glu Glu Asp Gly Ile Thr Trp Thr Ser Asp Gln	
50 55 60	
AGC AGT GAG GTC TTG GGC TCT GGC AAA ACC TTG ACC ATC CAA GTC AAA	240
Ser Ser Glu Val Leu Gly Ser Gly Lys Thr Leu Thr Ile Gln Val Lys	
65 70 75 80	
GAG TTT GGA GAT GCT GGG CAG TAC ACC TGT CAC AAA GGA GGC GAG GTC	288
Glu Phe Gly Asp Ala Gly Gln Tyr Thr Cys His Lys Gly Gly Glu Val	
85 90 95	
CTG AGT CGT TCA CTC CTC CTG CTC AAC AAA TAC GAA GAT GGA ATT TGG	336
Leu Ser Arg Ser Leu Leu Leu Leu His Lys Lys Glu Asp Gly Ile Trp	
100 105 110	

- 50 -

TCC ACT GAT ATT TTA AGG GAT CAG AAA GAA CCC AAA GCT AAG AGT TTT	384		
Ser Thr Asp Ile Leu Arg Asp Gln Lys Glu Pro Lys Ala Lys Ser Phe			
115	120	125	
TTA AAA TGT GAG GCA AAG GAT TAT TCT GGA CAC TTC ACC TGC TCG TGG	432		
Leu Lys Cys Glu Ala Lys Asp Tyr Ser Gly His Phe Thr Cys Ser Trp			
130	135	140	
CTG ACA GCA ATC AGT ACT AAT TTG AAA TTC AGT GTC AAA AGC AGC AGA	480		
Leu Thr Ala Ile Ser Thr Asn Leu Lys Phe Ser Val Lys Ser Ser Arg			
145	150	155	160
GGC TCC TCT GAC CCC CGA GGG GTG ACG TGC GGA GCA GCG TCC CTC TCA	528		
Gly Ser Ser Asp Pro Arg Gly Val Thr Cys Gly Ala Ala Ser Leu Ser			
165	170	175	
GCA GAG AAG GTC AGC ATG GAC CAC AGG GAG TAT AAC AAG TAC ACA GTG	576		
Ala Glu Lys Val Ser Met Asp His Arg Glu Tyr Asn Lys Tyr Thr Val			
180	185	190	
GAG TGT CAG GAG GGC AGC GCC TGC CCA GCC GCT GAG GAG AGC CTG CTT	624		
Glu Cys Gln Glu Gly Ser Ala Cys Pro Ala Ala Glu Glu Ser Leu Leu			
195	200	205	
ATT GAG GTC GTG ATG GAA ACT GTG CAC AAG CTC AAG TAT GAA AAC TAC	672		
Ile Glu Val Val Met Glu Thr Val His Lys Leu Lys Tyr Glu Asn Tyr			
210	215	220	
ACC AGC AGC TTC TTC ATC AGG GAC ATC ATC AAA CCA GAC CCA CCC AAG	720		
Thr Ser Ser Phe Phe Ile Arg Asp Ile Ile Lys Pro Asp Pro Pro Lys			
225	230	235	240
AAC CTG CAA CTG AGA CCA TTA AAG AAT TCT CGG CAG GTG GAG GTC AGC	768		
Asn Leu Gln Leu Arg Pro Leu Lys Asn Ser Arg Gln Val Glu Val Ser			
245	250	255	
TGG GAG TAC CCT GAC ACG TGG AGC ACC CCG CAT TCC TAC TTC TCC CTG	816		
Trp Glu Tyr Pro Asp Thr Trp Ser Thr Pro His Ser Tyr Phe Ser Leu			
260	265	270	
ACG TTT TGT GTT CAG GTC CAG GGA AAG AAC AAG AGA GAA AAG AAA CTC	864		
Thr Phe Cys Val Gln Val Gln Gly Lys Asn Lys Arg Glu Lys Lys Leu			
275	280	285	

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TTC ACA GAC CAA ACC TCA GCC AAA GTC ACA TGC CAC AAG GAT GCC AAC	912
Phe Thr Asp Gln Thr Ser Ala Lys Val Thr Cys His Lys Asp Ala Asn	
290	295
	300
ATC CGC GTG CAA GCC CGG GAC CGC TAC TAC AAC TCA TTC TGG AGT GAA	960
Ile Arg Val Gln Ala Arg Asp Arg Tyr Tyr Asn Ser Phe Trp Ser Glu	
305	310
	315
	320
TGG GCA TCT GTG TCC TGC AGT TAG	984
Trp Ala Ser Val Ser Cys Ser	
	325

(11) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 327 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Met His Pro Gln Gln Leu Val Val Ser Trp Phe Ser Leu Val Leu Leu			
1	5	10	15
Ala Ser Pro Ile Val Ala Ile Trp Glu Leu Glu Lys Asn Val Tyr Val			
20	25	30	
Val Glu Leu Asp Trp Tyr Pro Asn Ala Pro Gly Glu Thr Val Val Leu			
35	40	45	
Thr Cys Asp Thr Pro Glu Glu Asp Gly Ile Thr Trp Thr Ser Asp Gln			
50	55	60	
Ser Ser Glu Val Leu Gly Ser Gly Lys Thr Leu Thr Ile Gln Val Lys			
65	70	75	80
Glu Phe Gly Asp Ala Gly Gln Tyr Thr Cys His Lys Gly Gly Glu Val			
85	90	95	
Leu Ser Arg Ser Leu Leu Leu His Lys Lys Glu Asp Gly Ile Trp			
100	105	110	

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Ser Thr Asp Ile Leu Arg Asp Gln Lys Glu Pro Lys Ala Lys Ser Phe
115 120 125

Leu Lys Cys Glu Ala Lys Asp Tyr Ser Gly His Phe Thr Cys Ser Trp
130 135 140

Leu Thr Ala Ile Ser Thr Asn Leu Lys Phe Ser Val Lys Ser Ser Arg
145 150 155 160

Gly Ser Ser Asp Pro Arg Gly Val Thr Cys Gly Ala Ala Ser Leu Ser
165 170 175

Ala Glu Lys Val Ser Met Asp His Arg Glu Tyr Asn Lys Tyr Thr Val
180 185 190

Glu Cys Gln Glu Gly Ser Ala Cys Pro Ala Ala Glu Glu Ser Leu Leu
195 200 205

Ile Glu Val Val Met Glu Thr Val His Lys Leu Lys Tyr Glu Asn Tyr
210 215 220

Thr Ser Ser Phe Phe Ile Arg Asp Ile Ile Lys Pro Asp Pro Pro Lys
225 230 235 240

Asn Leu Gln Leu Arg Pro Leu Lys Asn Ser Arg Gln Val Glu Val Ser
245 250 255

Trp Glu Tyr Pro Asp Thr Trp Ser Thr Pro His Ser Tyr Phe Ser Leu
260 265 270

Thr Phe Cys Val Gln Val Gln Gly Lys Asn Lys Arg Glu Lys Lys Leu
275 280 285

Phe Thr Asp Gln Thr Ser Ala Lys Val Thr Cys His Lys Asp Ala Asn
290 295 300

Ile Arg Val Gln Ala Arg Asp Arg Tyr Tyr Asn Ser Phe Trp Ser Glu
305 310 315 320

Trp Ala Ser Val Ser Cys Ser
325

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(12) INFORMATION FOR SEQ ID NO:11:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

CTTTCTTTGC CAAAGGCAAA CGC

23

(13) INFORMATION FOR SEQ ID NO:12:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

TGGCCCTCAT TCTCACTGCA

20

(14) INFORMATION FOR SEQ ID NO:13:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

CGCGGATCCA TGCATCTGCG TTTGACCTTG

30

- 54 -

(15) INFORMATION FOR SEQ ID NO:14:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

TCAGCTTCC ATGCTCCACT C

21

(16) INFORMATION FOR SEQ ID NO:15:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 29 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

CGGGATCCA CCACCTCAGT TTGCCAGG

29

(17) INFORMATION FOR SEQ ID NO:16:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

CGCGGATCCG GCGTGAAGCA GGATGCAGAG

30

(18) INFORMATION FOR SEQ ID NO:17:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 30 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

CGCCTCGAGA TGTGCCCGCT TCGCAGCCTC

30

(19) INFORMATION FOR SEQ ID NO:18:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 30 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

CGCGGTACCC TAGGAAGAAC TCAGATAGCT

30

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(20) INFORMATION FOR SEQ ID NO:19:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 30 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

CGCGGATCCA TGCACCCCTCA GCAGTTGGTC

30

(21) INFORMATION FOR SEQ ID NO:20:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 30 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

CGCGTCGACA CTGCAGGACA CAGATGCCA

30

CLAIMS:

1. An isolated nucleic acid molecule comprising a nucleotide sequence encoding or complementary to a nucleotide sequence encoding an ovine cytokine or a functional or 5 immunologically interactive homologue, analogue or derivative thereof, wherein said cytokine is IL-5.
2. An isolated nucleic acid molecule comprising a nucleotide sequence encoding a complementary to a nucleotide sequence encoding an ovine cytokine or a functional or 10 immunologically interactive homologue, analogue or derivative thereof, wherein said cytokine is IL-12 or a polypeptide subunit of IL-12 as hereinbefore defined.
3. The isolated nucleic acid molecule according to claim 2, wherein the cytokine is a fusion cytokine between different subunits of IL-12.
15
4. The isolated nucleic acid molecule according to any one of claims 1 to 3 wherein the nucleotide sequence comprises deoxyribonucleotides.
5. The isolated nucleic acid molecule according to claim 4 wherein the nucleotide 20 sequence is a double-stranded cDNA or synthetic DNA molecule.
6. The isolated nucleic acid molecule according to claim 1 or 3 wherein said IL-5 cytokine further comprises a nucleotide sequence set forth in any one of SEQ ID Nos: 1 or 3 or a homologue, analogue or derivative thereof.
25
7. The isolated nucleic acid molecule according to claim 2 or 3 wherein said IL-12 subunit is a 35 kDa IL-12 polypeptide.
8. The isolated nucleic acid molecule according to claim 7 wherein said IL-12 subunit 30 further comprises a nucleotide sequence set forth in any one of SEQ ID Nos: 5 or 7 or a

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homologue, analogue or derivative thereof.

9. The isolated nucleic acid molecule according to claim 2 or 3 wherein said IL-12 subunit is a 40 kDa IL-12 polypeptide.

5

10. The isolated nucleic acid molecule according to claim 9 wherein said IL-12 subunit further comprises a nucleotide sequence set forth in SEQ ID No: 9 or a homologue, analogue or derivative thereof.

10 11. An isolated DNA molecule which encodes a molecule having interleukin activity and is capable of hybridising under at least medium stringency conditions as hereinbefore defined to one or more of SEQ ID Nos: 1, 3, 5, 7 or 9 or a complementary sequence or a homologue, analogue or derivative thereof, wherein said interleukin comprises an amino acid sequence which corresponds or is at least 70% identical to all or a functional or 15 immunologically-interactive part of any one of SEQ ID Nos: 2, 4, 6, 8 or 10.

12. The isolated DNA molecule according to claim 11 wherein said interleukin is ovine IL-5.

20 13. The isolated DNA molecule according to claim 11 wherein said interleukin is ovine IL-12.

14. A genetic construct which comprises a nucleic acid molecule which encodes or is complementary to a nucleic acid molecule which encodes an ovine IL-5 polypeptide or a 25 homologue, analogue or derivative thereof.

15. The genetic construct according to claim 14 wherein said nucleic acid molecule comprises a sequence of nucleotides set forth in SEQ ID No: 1 or SEQ ID No: 3 or a homologue, analogue or derivative thereof.

16. The genetic construct according to claim 14 wherein said nucleic acid molecule encodes an IL-5 polypeptide comprising an amino acid sequence set forth in SEQ ID No. 2 or 4 or is at least 70% identical thereto.

5 17. A genetic construct which comprises a nucleic acid molecule which encodes or is complementary to a nucleic acid molecule which encodes an ovine IL-12 polypeptide or a homologue, analogue or derivative thereof.

10 18. The genetic construct according to claim 17 wherein said nucleic acid molecule comprises a sequence of nucleotides set forth in SEQ ID No: 5 or SEQ ID No: 7 or a homologue, analogue or derivative thereof.

15 19. The genetic construct according to claim 17 wherein said nucleic acid molecule encodes an IL-12 polypeptide which has an estimated molecular weight of approximately 35 kDa, as determined using SDS/PAGE.

20 20. The genetic construct according to claim 19 wherein said nucleic acid molecule encodes an IL-12 polypeptide further comprising a sequence of amino acids set forth in SEQ ID No: 6 or SEQ ID No: 8 or is at least 80% identical thereto.

21. The genetic construct according to claim 17 wherein said nucleic acid molecule comprises a sequence of nucleotides set forth in SEQ ID No: 9 or a homologue, analogue or derivative thereof.

25 22. The genetic construct according to claim 17 wherein said nucleic acid molecule encodes an IL-12 polypeptide having an estimated molecular weight of approximately 40 kDa as determined using SDS/PAGE.

30 23. The genetic construct according to claim 22 wherein said nucleic acid molecule further encodes an IL-12 polypeptide comprising a sequence of amino acids set forth in

- 60 -

SEQ ID No: 10 or is at least 80% identical thereto.

24. A genetic construct which comprises a sequence of nucleotides which is capable of hybridising under at least medium stringency conditions as hereinbefore defined to any one
5 of the ovine IL-5 or IL-12 nucleotide sequences set forth in SEQ ID Nos: 1, 3, 5, 7 or 9 or a complementary sequence or a homologue, analogue or derivative thereof.

25. The genetic construct according to any one of claims 14 to 24 further comprising a promoter sequence operably linked to said nucleic acid molecule or sequence of nucleotides.

10

26. The genetic construct according to claim 25 wherein said promoter is suitable for expression in a bacterial cell.

27. The genetic construct according to claim 26 wherein said promoter is the *tac* promoter, *lacZ* promoter or phage lambda λ_c or λ_r promoter sequence.
15

28. The genetic construct according to claim 25 wherein said promoter is suitable for expression in a eukaryotic cell.

20 29. A recombinant isolated ovine IL-5 polypeptide or a homologue, analogue or derivative thereof.

30. A recombinant isolated ovine IL-12 polypeptide or a homologue, analogue or derivative thereof.

25

31. The recombinant polypeptide according to claim 29 or 30 wherein said polypeptide comprises a sequence of amino acids set forth in any one of SEQ ID Nos: 2, 4, 6, 8 or 10 or is at least 70% identical thereto or a derivative thereof.

30 32. A method for the treatment and/or prophylaxis of a livestock animal which has been

exposed to or infected with a pathogenic organism, said method comprising administering to said animal an immunoresponsive effective amount of ovine IL-5 and IL-12 or a homologue, analogue or derivative thereof for a time and under conditions sufficient to maintain, stimulate or enhance the immunoresponsiveness of said animal.

5

33. The method of claim 32 wherein the ovine cytokine is a recombinant molecule.
34. The method according to claim 33 wherein the recombinant molecule is according to claim 31.
- 10 35. The method according to claim 32 or 33 or 34 wherein the animal is selected from the list comprising sheep, horses, pigs, cows, donkeys, emus, ostriches, alpacas, camels, deer and goats.
- 15 36. The method according to claim 35 wherein the animal is a sheep.
37. The method according to claim 35 wherein the animal is a cow.
38. The method according to any one of claims 32 to 37 further comprising the 20 administration of one or more antigens.
39. A vaccine comprising a recombinant ovine IL-5 or IL-12 molecule or a homologue, analogue or derivative thereof and an antigen.
- 25 40. The vaccine according to claim 39 wherein the recombinant molecule comprises a sequence of amino acids set forth in any one of SEQ ID Nos: 2, 4, 6, 8 or 10 or is at least 70% identical thereto.
- 30 41. The vaccine according to claims 39 or 40 further comprising a pharmaceutically

acceptable carrier or diluent.

42. The vaccine according to any one of claims 39 to 41, suitable for veterinary use.
- 5 43. A genetic construct comprising a first nucleotide sequence encoding ovine IL-5 or ovine IL-12 or a derivative thereof and a second nucleotide sequence comprising a delivery vehicle which is capable of controlling replication in a bacterial, yeast, insect, protozoan animal or a mammalian cell.
- 10 44. The genetic construct according to claim 43 wherein the first nucleotide sequence comprises a sequence of nucleotides set forth in any one of SEQ ID Nos: 1, 3, 5, 7 or 9 or a homologue, analogue or derivative thereof.
- 15 45. The genetic construct according to claim 43 or 44 wherein the first nucleotide sequence is linked to a promoter sequence which is capable of regulating expression of said nucleotide sequence in the same cell in which the delivery vehicle is capable of controlling replication.
- 20 46. A delivery cell comprising the genetic construct of claim 45.
47. The delivery cell according to claim 46 wherein said cell is a bacterial cell or an attenuated virus.
- 25 48. The method according to claim 32 wherein the step of administering an ovine IL-5 or IL-12 molecule is by means of a genetic construct according to any one of claims 43 to 45 or a delivery cell according to claim 46 or 47.
- 30 49. The method according to claim 32 wherein the step of administering an ovine IL-5 or IL-12 molecule is by injection.

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50. A veterinary pharmaceutical composition comprising a recombinant ovine IL-5 or IL-12 polypeptide or a derivative thereof and one or more carriers and/or diluents suitable for veterinary use.

51. The composition according to claim 50 wherein the recombinant ovine IL-5 or IL-12 polypeptide comprises a sequence of amino acids substantially the same as any one of SEQ ID Nos: 2, 4, 6, 8 or 10 or is at least 70% identical thereto.

52. An antibody molecule capable of binding to a recombinant ovine IL-5 or IL-12 polypeptide or a derivative thereof.

53. The antibody molecule according to claim 52 further capable of binding to an IL-5 or IL-12 polypeptide which comprises a sequence of amino acids substantially the same as any of SEQ ID Nos: 2, 4, 6, 8 or 10 or is at least 70% identical thereto.

15

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FIGURE 1

10 20 30 40 50 60
 CTTTCTTGCCAAAGGCAAACGCTGAACATTCAGAGTCAGAGAATGCATCTGCGTTG
 M H L R L
 [-----]

70 80 90 100 110 120
 ACCTTGGTAGCTCTTGGAGCTGCCATGTTGTGCCATGCTGTAGAAAGTACCATGAAT
 T L V A L G A A Y V C A N A V E S T M N
 ----- exon 1 -----

130 140 150 160 170 180
 AGACTGGTGGCAGAGACCTTGACACTGCTCCACGCATCAAACCTCTGCTGATAGGTGAT
 R L V A E T L T L L S T H Q T L L I G D
 ----- exon 1 -----

190 200 210 220 230 240
 GGGAACTTGATGATTCCTACTCCTCAGCATACAAATCACCAACTATGCATTGAAGAACGTC
 G N L M I P T P Q H T N H Q L C I E E V
 --II-----exon 2 -----II-----exon 3-----

250 260 270 280 290 300
 TTTCAGGAAATAGACACATTGAAGAATCAAACCTGACAAGGGGATGCTGTGAAAAAAAATA
 F Q G I D T L K N Q T A Q G D A V K K I
 ----- exon 3 -----

310 320 330 340 350 360
 TTCCGAAACTTGCTTTAATAAAAAGAATAACATAGACCTCCAAAAAAAGGAAGTGTGAGGAGA
 F R N L S L I K E Y I D L Q K R K C G G
 ----- exon 3 -----II-----exon 4 -----

370 380 390 400 410 420
 GAAAGATGGAGAGTGAACAAATTCCCTCGACTACCTGCAAGTTTCTTGGTGTGATAAAC
 E R W R V K Q F L D Y L Q V F L G V I N
 ----- exon 4 -----

430 440 450 460 470 480
 ACAGAGTGGACGATGGAAAGCTGAGATCTACCTCTCACTGTAGTGAAGTTCTGGAG
 T E W T M E S *
 ----- exon 4 -----

490 500 510 520
 GAGGAGAAGGATGTTTAATTGCACTCAGAATGAGGGCCA 3'

FIGURE 2

Exon 1

	10	20	30	40
Ovine	WHLRETLVRLGAYVCAANAVESTNNRIVAEITLILSTQGFLLYGDG			
Human	M.RML-H-S-L-----Y-IPT-IPTSA--K---A---R---ANZ			
Mouse	MRRML-H-SVLT..SC-W-T-M-IP-STV-K---Q-SA-RA--TSNZ			

Exon 2

	47
Ovine	NLMIPTPQHIN
Human	T-R--V-V-K-
Mouse	TMRL-V-T-K-

Exon 3

	58	70	80	90	100
Ovine	HQLCIEEVFPQGIDILKNQTAQGDAVKKIPRNLSLIKELYIDLQK				
Human	----T--I----G--ES--V--GR-ERL-K-----K--G--				
Mouse	----G-I---L-I----VR-GT-EML-Q-----K---R--				

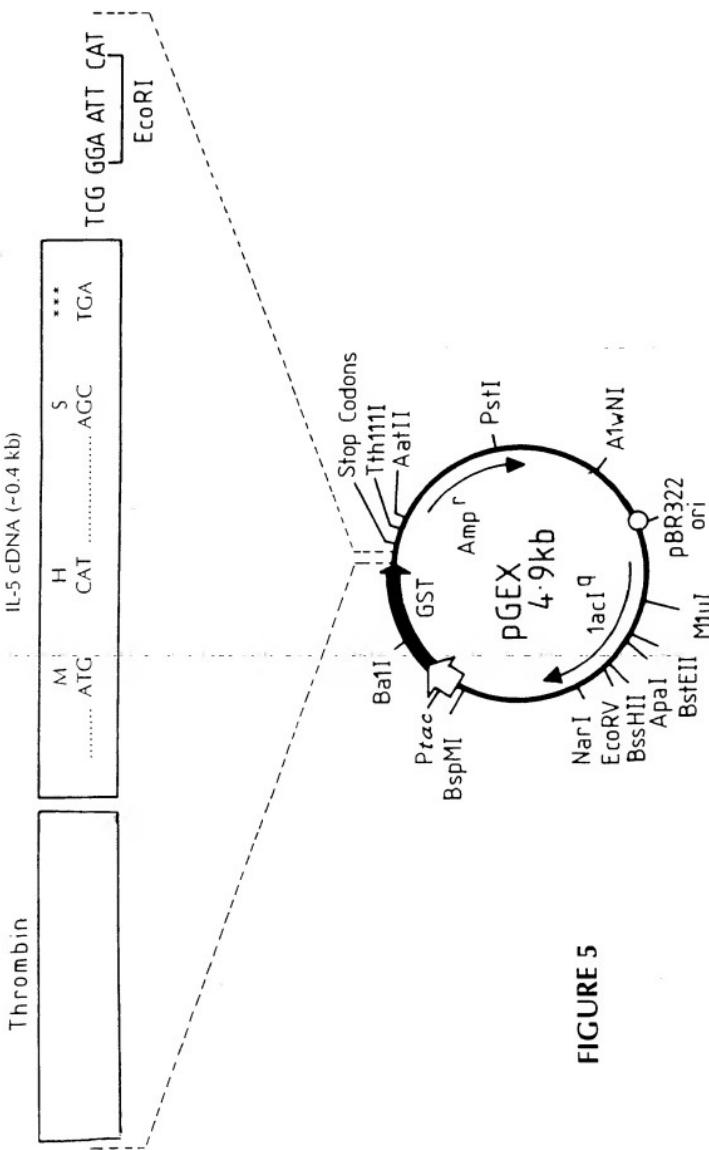
Exon 4

	101	132
Ovine	RKCGGERWRVKQFLDYFLQVFGLVINTEWTHES	
Human	K---E--R--N-----E---H---II--	
Mouse	E---E--R-TR-----E---MS---A-G	

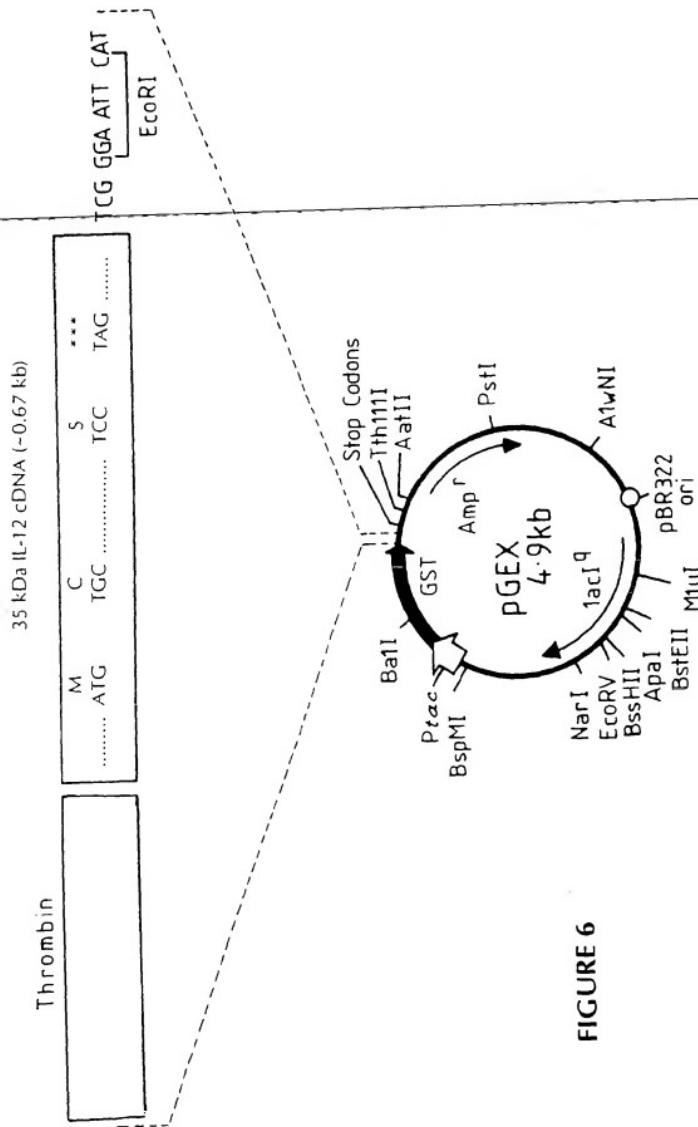
3/9

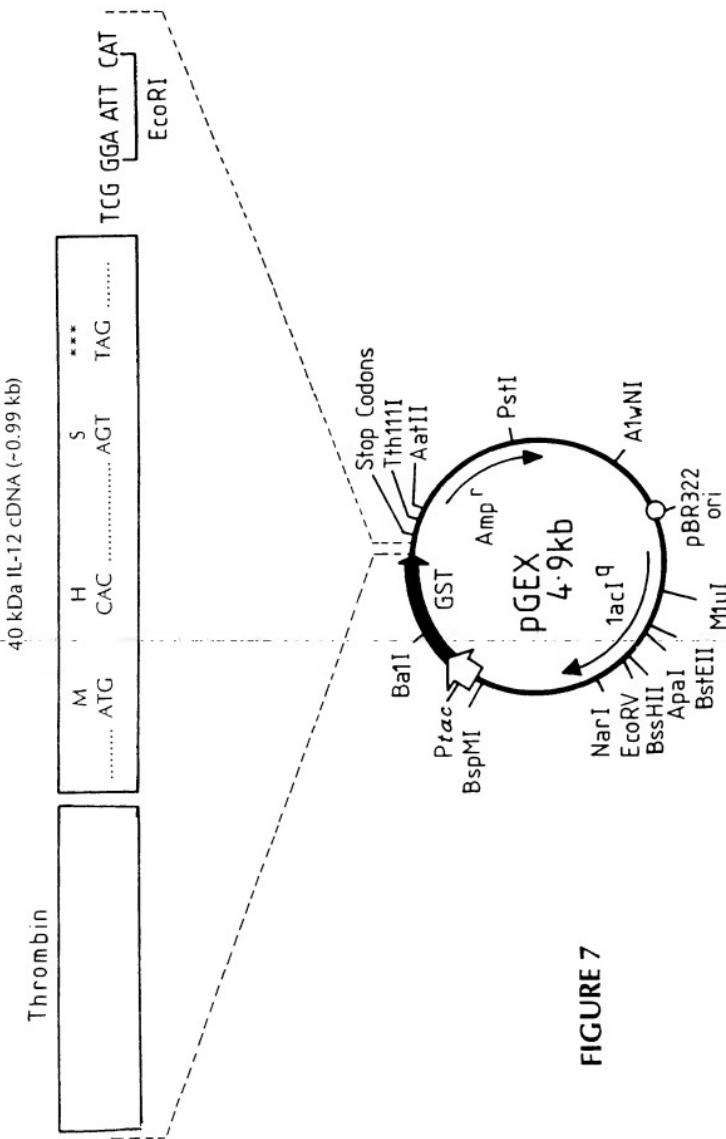
FIGURE 3

	10	20	30	40	50	60
ovine	HHL	SLARSLPTTTAGPGR.	SCLDYSQNL	LLRAVSNTL	QKARQTL	EFLYSC
bovine	PHL	SLGRSLPTTTASPGR.	SCLDYSQNL	LLRAVSNTL	QKARQTL	EFLYSC
human	DHL	SLARNLPVATPDGMP	PCLHHSQNL	LLRAVS	NHLQKARQ	TLEFLYPC
mouse	NHL	SLARVIP..VSGPAR..	CLSQS	RNL	LKTDDMV	KTA
	70	80	90	100	110	120
ovine	KTSTVEAC	PLPLELATNES	CLASRET	SLITNGH	CLSSGKTS	FMTTL
bovine	KTSTVEAC	PLPLELATNES	CLASRET	SFITNGH	CLASGKTS	FMTTL
human	KTSTVEAC	PLPLELTKNES	CLNSRET	SFITNGS	CLASRKTS	FHMALCL
mouse	QTSTLKTC	PLPLELHRN	ESCLATRET	SSTTRGS	CLPPQKTS	LMHTLCLGSI
	130	140	150	160	170	180
ovine	FQAMNAK	LLMDKRQVFLDQ	NMLAAIAELM	HQALNF	DFSETVPQ	KPSLEELDFY
bovine	FQAMNAK	LLMDKRQI	FLDQNM	LAIAELM	QALNFDSETVP	QKPSLKE
human	FKTMNAK	LLMDKRQI	FLDQNM	LAVIDELM	QALNFNSE	TPQKSSL
mouse	FQAINA	ALQNHQQI	LDKGML	VAIDELM	QSLNHNG	ETLRQKPPVG



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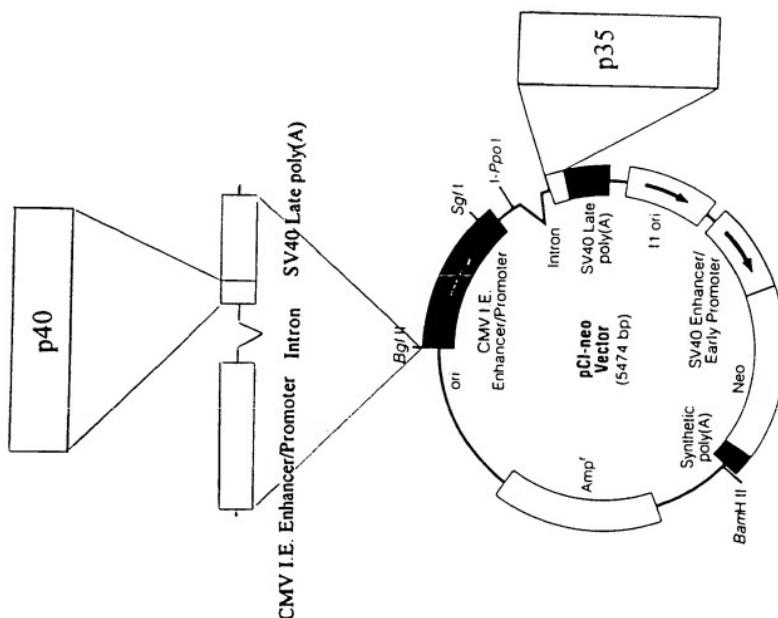


FIGURE 8

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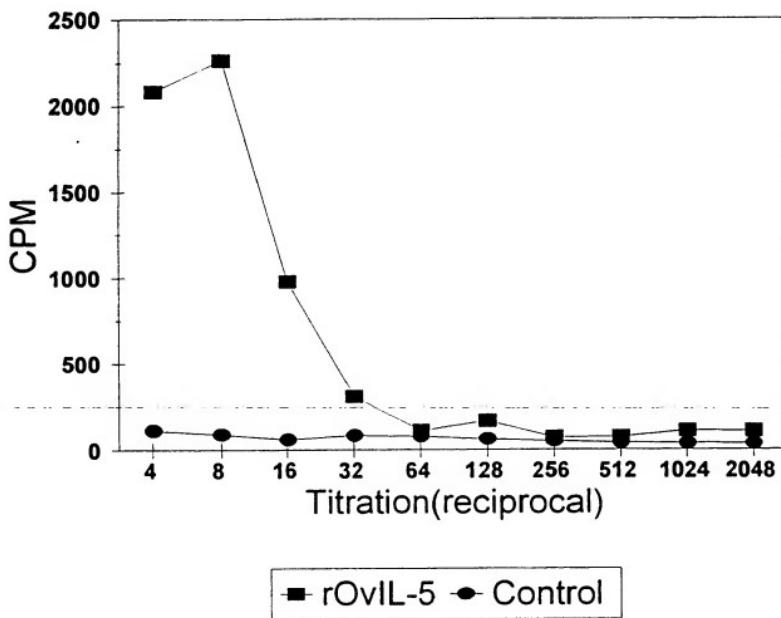


FIGURE 9

INTERNATIONAL SEARCH REPORT

International Application No.
PCT/AU 96/00360

A. CLASSIFICATION OF SUBJECT MATTER

Int Cl⁶: C12N 15/24; C07K 16/24; C12N 15/62, 5/10; A61K 38/20, 48/00.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

WPAT AND CHEM ABS

SEE DETAILS IN ELECTRONIC DATABASE BOX BELOW

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPM AND JAPIO

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
DERWENT WPAT, USPM, JAPIO DATA BASES; KEYWORDS: (INTERLEUKIN()5 OR IL()5 OR ILS OR EOSINOPHIL() DIFFERENTIATION() FACTOR OR EDF OR EOSINOPHIL() COLONY() STIMULATING() FACTOR OR T() CELL() REPLACING() FACTOR OR EO() CSF OR KHF OR IGA() EF) AND (C12N-015/1C OR A61k/1C).
CHEMICAL ABSTRACTS DATABASE; KEYWORDS: (GENE# OR GENET?)IT AND INTERLEUKIN()5/IT AND 1991-1996/PY.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	J Biochem (1990), Vol. 107, Pages 292-297. (Minamitake, Y. et al) "Structure of Recombinant Human Interleukin 5 Produced by Chinese Hamster Ovary Cells". See whole Article.	1,4-6,11,12, 14-16, 24-29 and 31-53.
Y		

Further documents are listed in the continuation of Box C

See patent family annex

* Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A" document defining the general state of the art which is not considered to be of particular relevance		
"E" earlier document but published on or after the international filing date	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)		
"O" document referring to an oral disclosure, use, exhibition or other means	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"P" document published prior to the international filing date but later than the priority date claimed	"&"	document member of the same patent family

Date of the actual completion of the international search
13 August 1996

Date of mailing of the international search report
27 AUG 1996

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PCT/INTERNATIONAL SEARCH REPORT

International Application No.
PCT/AU 96/00360

C (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	GB 2217328 (British Bio-Technology Limited) published 25 October 1989. See whole document	1,4-6,11,12, 14-16,24-29 and 31-53
X	EP 621341 (American Cyanamid Co.) published 26 October 1994. See whole document	52 and 53
X	Cytokine, (January 1991), Vol. 3, No. 1, Pages 72-81.(Uberla, K. et al.) "The Rat Interleukin-5 Gene: Characterization and Expression by Retroviral gene transfer and Polymerase chain reaction".	1,4-6,11,12 14-16,24-29 and 31-53
Y	AU 85278/91 (Commonwealth Scientific and Industrial Research Organization) published 2 April 1992. See whole Article.	1,4-6,11,12, 14-16,24-29 and 31-53

INTERNATIONAL SEARCH REPORT

International Application No.

PCT/AU 96/00360

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely:

2. Claims Nos.: 1, 6, 11, 14-16, 24, 29, 31, 32, 39, 40, 44 and 50-53 because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
Parts of the above claims referring to homologues, derivatives, sequences 70% identical to the parent sequence, and immunologically-interactive parts were found to be unsearchable because they are indeterminate in scope and are not the biological equivalents of ovine IL-5.

3. Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a)

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

The International Searching Authority has found that there are two inventions:

1. Claims 1, 4-6, 11, 12, 14-16, 24-29 and 31-53 are directed to isolated nucleic acid molecule of Ovine Interleukin 5. Genetic constructs, vaccines, pharmaceutical compositions comprising it. Delivery cells comprising the genetic constructs. Method of treating animals with ovine Interleukin 5 and antibodies to IL-5. This comprises a "first special technical feature".

continued

1. As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims
2. As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: 1, 4-6, 11, 12, 14-16, 24-29 and 31-53

Remark on Protest

 The additional search fees were accompanied by the applicant's protest. No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International Application No.

PCT/AU 96/00360

Box II continuation

2. Claims 2-11, 13, 17-28, 30-53 are directed to isolated nucleic acid molecule of ovine Interleukin-12. Genetic constructs, vaccines, pharmaceutical compositions comprising it. Delivery cells comprising the genetic constructs. Method of treating animals with ovine Interleukin-12 and antibodies to IL-12. This comprises a second separate "special technical feature".

The two sets of claims do not share a technical relationship because Interleukin-5 and Interleukin-12 are structurally distinct molecules with different biological functions and unrelated genes.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No.
PCT/AU 96/00360

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report				Patent Family Member		
GB	2217328	GB	8808524			
EP	621341	AU	60591/94	CA	2121096	JP
AU	A 91 85278	NZ	239791	WO	9205255	

END OF ANNEX

